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Fifteen New Species of the Genus *Hemicycliophora* with an Emended Description of *H. typica* de Man (Tylenchida Criconematidae)

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The recent work on *Hemicycliophora* by Tarjan, 1952, appeared in these "Proceedings" while the writer was engaged on a similar task. Tarjan's excellent review of the previous history of the genus adequately covers the species already described.

Because of the rapidly developing interest in nematodes ectoparasitic on plants, it appears advisable to present the descriptions of the fifteen new species which have been assembled at the Salt Lake City Station of the Section of Nematology and in the collection of the University of California at Berkeley. Through the kindness of Dr. M. W. Allen, a collection of *Hemicycliophora typica* de Man, 1921, from the type locality in Holland was made available and the description is herein emended. These specimens show that the females do not have longitudinal striae and therefore *H. membranifer* Micoletzky is a valid species and not synonymous with *H. typica*, as Loos, 1948, thought. Dr. S. A. Sher contributed his collection from which *H. uniformis* n. sp. is described. Mr. Clive A. Loos of the Tea Research Institute of Ceylon kindly loaned specimens of *H. longicaudata* Loos, and re-examination of this species reveals that it belongs to an undescribed genus that combines characters of both *Hemicycliophora* and *Criconemoides*.

Records of distribution of the present known species indicate that they prefer soils about the roots of plants growing in moist locations, especially in forests and along stream banks. However, as will be noted, some of them are inhabitants of cultivated fields and greenhouse soils where they doubtless feed on the roots of economically important plants.

Tarjan found *H. parvana* Tarjan in cultivated soil in Florida and later reproduced it on celery, *Apium graveolens*, under greenhouse conditions. *H. obtusa* n. sp. was found about the roots of sugar beets near Lewiston, Utah, and *H. similis* n. sp. inhabited an alfalfa field in Nevada, a peach orchard in California and rose beds in a greenhouse near Denver, Colorado. *H. penetrans*, n. sp. was abundant in rice and corn plantings on the Experiment Station, Bogor, Java, and *H. conida* n. sp. was found in three sugar beet fields in Ireland.

Only relatively small populations have been recorded for any of these species and their possible importance has not been determined. Steiner 1949 (Fig. 24) illustrates *Procriconema* sp. (synonym of *Hemicycliophora*) in considerable numbers attacking roots of slash pine seedlings. We know that also other members of the CRICONEMATIDAE are associated with certain types of dieback or decline in orchards, and we are justified in suspecting any

of the ectoparasitic root-feeding species, especially those with well developed spears like those of *Hemicycliophora* and *Criconemoides*. *Cacopaurus pestis* Thorne is recognized as the causal agent of a dieback in walnuts, (Thorne, 1943), and *Paratylenchus hamatus* Thorne and Allen is associated with a premature leaf and fruit drop in figs, (Thorne and Allen, 1950), and Lownsbery *et al*, 1952, report it as infesting celery. Jackson, 1948, reported *Criconemoides rusticum* (Micoletzky) Taylor as involved with the deterioration of shortleaf pine roots, *Pinus echinata*, and Machmer, 1953, records *Criconemoides* sp. as a "major association with peanut yellows." Linford, Oliveira and Ishii, 1949, described *Paratylenchus minutus*, a parasite attacking roots of various plants in Hawaii.

The fact that only small populations of *Hemicycliophora* have been found does not indicate that they may be of only minor importance, for even very low populations of ectoparasitic nemas may cause extensive damage, as Christie and Perry, 1951, discovered when *Trichodorus* sp. was definitely determined as the causal agent of "stubby-root" in celery, sweet corn and other crops in Florida.

One of the most interesting points developed during this study of *Hemicycliophora* concerns the hemizonid, a structure named by J. Basil Goodey in 1951. This ventrally located organ extends between the lateral fields just underneath the cuticle, and usually is observed as a refractive, crescentic marking near the excretory pore. The hemizonid is present in both males and females of *H. typica* and *H. penetrans*, the only two species of *Hemicycliophora* known to be bisexual. Very obscure hemizonids were seen in a few of the 16 specimens of *H. obesa* n. sp., a species in which a spermatheca is present but spermatozoa were not observed. The single specimen of *H. tenuis* n. sp. possesses an easily defined hemizonid but neither a spermatheca nor spermatozoa were present. The writer failed to find hemizonids in the other 12 monosexual species studied, and Tarjan observed that he was unable to locate a hemizonid in *H. parvana*. These combined points of evidence indicate that in some manner the hemizonid may possibly be associated with the sexual processes.

The rectum and anus are always difficult to locate accurately and therefore the tail measurements have been omitted in the formulae. However, the term "tail" is used as a matter of convenience to designate the tapering posterior portion of the body which is very important in species identification.

Illustrations are of a uniform magnification of approximately 400 diameters except those of the lateral fields of *gigas, penetrans, conida, gracilis* and *typica* which are about 600 diameters.

KEY TO FEMALES OF *Hemicycliophora*

1. Terminus acute or subacute 2
   Terminus blunt, rounded 16
2. Length about 0.4 mm. ..... *straeleni* de Coninck, 1931
   Length 0.7 mm or more 3
3. Tail long, attenuated 4
   Tail not attenuated 5
4. Neck cylindroid to truncate lip region ..... *micoletzki* (Goffart, 1948) Goffart, 1952
   Neck tapering to rounded lip region ..... *gigas* n. sp.
5. Body slender, a = 33 — 36 6
   Body more robust, a = 19 — 26 7

6. Length 1.0 mm, body arcuate ..... *arcuata* n. sp.
   Length 1.4 mm, body not arcuate ..... *tenuis* n. sp.
7. Body marked by numerous longitudinal striae 8
   Body not marked by longitudinal striae 9
8. Tail concave conoid ..... *membranifer* Micoletzky, 1925
   Tail uniformly conoid ..... *penetrans* n. sp.
9. Cuticle ornamented with delicate longitudinal markings ..... *aquaticum* (Micoletzky, 1913) Loos, 1948
   Cuticle not ornamented with longitudinal markings 10
10. Body tapering rather uniformly from vulva to sub-acute terminus 11
    Body not tapering uniformly, tail concave conoid 13
11. Vulva-terminus distance three times spear length ..... *parvana* Tarjan, 1952
    Vulva-terminus distance twice spear length or less 12
12. Lip region broad, truncated ..... *conida* n. sp.
    Lip region narrowed, rounded ..... *uniformis* n. sp.
13. Length near 1.2 mm 14
    Length near 1.7 mm ..... *gracilis* n. sp.
14. Bisexual species with many males ..... *typica* de Man, 1921
    Monosexual species as far as is known 15
15. Body narrowing rapidly behind vulva ..... *thienemanni* (Schneider, 1922,[1] 1925) Loos, 1948
    Body slightly narrowed ventrally behind vulva ..... *similis* n. sp.
16. Terminus convex or irregular conoid to blunt terminus 17
    Terminus hemispheriod 20
17. Tail uniformly convex conoid 18
    Tail irregularly conoid to blunt terminus 19
18. Vulva-terminus distance less than spear length ..... *brevis* n. sp.
    Vulva-terminus distance one and one-half times spear length ..... *obesa* n. sp.
19. About 20 annules between vulva and terminus ..... *aberrans* n. sp.
    About 55 annules between vulva and terminus ..... *striatula* n. sp.
20. Length 1.2 mm ..... *rotundicauda* n. sp.
    Length about 0.8 mm 21
21. Body with ventral contraction at vulva ..... *obtusa* n. sp.
    Vulva continuous with body contour ..... *nana* n. sp.

Hemicycliophora gigas n. sp. (Fig. 1)

Female: 1.9 mm; a = 27; b = 5.5; V — 72

Larval cuticle fitting closely about the body throughout its length. Lip region rounded with the labial disc slightly elevated above the head contour. Body ending in a convex-conoid attenuated tail which becomes very slender near the pointed terminus. Cuticle marked by about 300 annules which are of rather uniform width except on the tail. On the lateral fields the crests of the annules are thinner and underneath the borders of the field are two series of ovate shadowy markings which may very easily be overlooked. These markings extend from close to the lip region to near the middle of the tail. Spear slightly arcuate, 150 μ long, bearing strongly developed basal knobs. Details of esophagus, intestine and reproductive system obscured by the dense body contents. Body slightly contracted ventrally at the depressed vulva.

[1] *Nomen nudum.* name without description or figure.

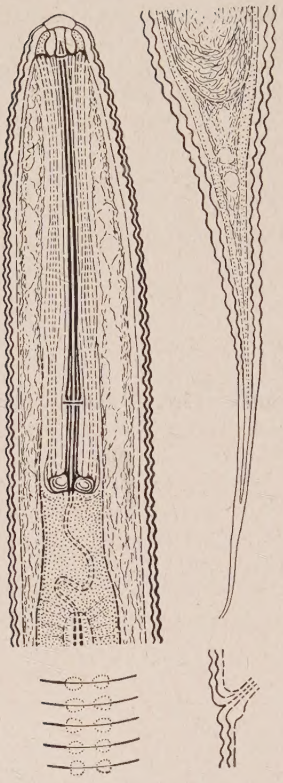

Fig. 1.—*Hemicycliophora gigas* n. sp.

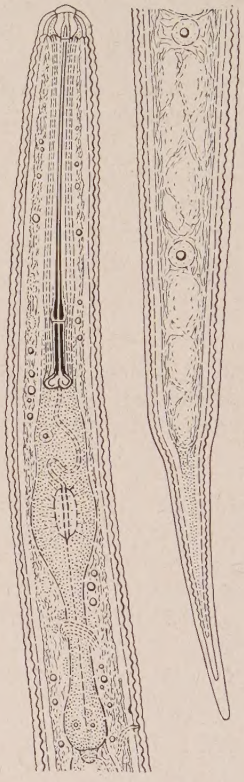

Fig. 2.—*Hemicycliophora tenuis* n. sp.

DIAGNOSIS.—*Hemicycliophora* with the above measurements and general description. Distinctive among the slender-tailed species because of its greater total length and the ovate shadowy markings of the cuticle of the lateral fields.

TYPE LOCALITY.—Clay forest soil, Duke University, Raleigh, North Carolina. Two females collected by A. S. Pearse.

TYPE SPECIMEN.—Largest of 2 ♀ on slide *Hemicycliophora* 5. NS-U (Collection of Nematology Section, Salt Lake City, Utah).

Hemicycliophora tenuis n. sp. (Fig. 2)

Female: 1.4 mm; a = 36; b = 5.2; V — $^{38}_{81}$

Body marked by 410 annules. Larval cuticle snugly fitting about body. On the posterior portion the annules disappear on the clinging larval cuticle. Lateral fields plain without longitudinal lines. Lip region without annulation. Labial disc elevated, about one-third as wide as lip region. Spear extending through 34 annules, its basal knobs rounded. Basal bulb of esophagus clavate. Nerve ring encircling middle of isthmus. Excretory pore about opposite base of esophagus. Hemizonid prominent, located one annule anterior to excretory pore. Intestinal cells vacuolated, each with a very large,

prominent nucleus. Ovary outstretched, slightly less than one-half as long as body. Uterus apparently without spermatheca. Vulva a depressed slit. There is no ventral contraction in the vulvar region. Anus not observed. Body tapering uniformly for three-fourths the distance behind the vulva, then constricted to form the uniformly conoid tail which ends in a small rounded point.

DIAGNOSIS.—*Hemicycliophora tenuis* is distinctive because of its slenderness, and its tail form.

TYPE LOCALITY.—A single specimen from stream bank soil, Little Cottonwood Canyon, near Salt Lake City, Utah.

TYPE SPECIMEN.—Female on slide *Hemicycliophora* 8, NS-U.

Hemicycliophora penetrans n. sp. (Fig. 3)

Female: 1.0 mm; a = 25; b = 6.0; V — $^{51}_{83}$

Body marked by about 256-280 annules. Larval cuticle fitting rather loosely around the body. Longitudinal lines mark the cuticle, about 20 being present at mid-body. On the lateral field two of these lines form a band of rectangular blocks about one-fourth as wide as the body. Lip region rounded, marked by 2 obscure annules. Labial disc about one-third as wide as head. Spear extending through 20-24 annules, its basal knobs projecting somewhat backward. Basal bulb of esophagus usually slightly clavate but sometimes continuous with the contour of the isthmus. Nerve ring varying in position from near the middle of the isthmus to the base of esophagus. Hemizonid conspicuous, 2-4 annules anterior to the exretory pore. Uterus with a very prominent spermatheca. Vulva located 41-53 annules in front of terminus. A long sheath attaches the vulva to the last larval cuticle.

Male: 0.8 mm; a = 28; b = 6.2; c = 7.3; T — 31

Body practically cylindrical, marked by rather fine striae. Lateral fields bordered by 2 conspicuous lines, leaving a plain narrow band between them. Lip region rounded, slightly expanded, without annulation. Pharynx with slightly sclerotized walls which sometimes appear to form an open chamber. Spear absent. Esophagus a slender tube extending back to the intestine. Hemizonid 3 or 4 annules anterior to the excretory pore, marked externally by a slight elevation of the cuticle. Intestinal cells vacuolated, almost devoid of granules. Testis outstretched. Spicula reflexed to a most unusual degree. The distal portion of the spicula are carried outside the body, and in two instances were partly broken off. Apparently nature has produced this unusual type of spicula in order to permit copulation through the long sheath leading from the vulva to the outside. Bursa broad, 3 or 4 times as long as the body width, folded over ventrally and covering the spicula when the nema is moving. Tail somewhat irregular—conoid to the fine rounded terminus.

DIAGNOSIS.—*Hemicycliophora penetrans* is distinctive because of the longitudinal lines marking the cuticle, the very slight clavate esophagus base, the long tube leading from the vulva and through the unshed larval cuticle, and the unusual reflexed type of spicula.

TYPE LOCALITY.—Several hundred specimens, including numerous males, from rice and corn plots, Experiment Station, Bogor, Java.

TYPE SPECIMEN.—Female No. 6 on slide *Hemicycliophora* 11, NS-U.

ALLOTYPE: Male on slide *Hemicycliophora* 11 a, NS-U.

Hemicycliophora arcuata n. sp. (Fig. 4)

Female: 0.65 mm; a = 33; b = 5.2; V — $^{53}_{81}$

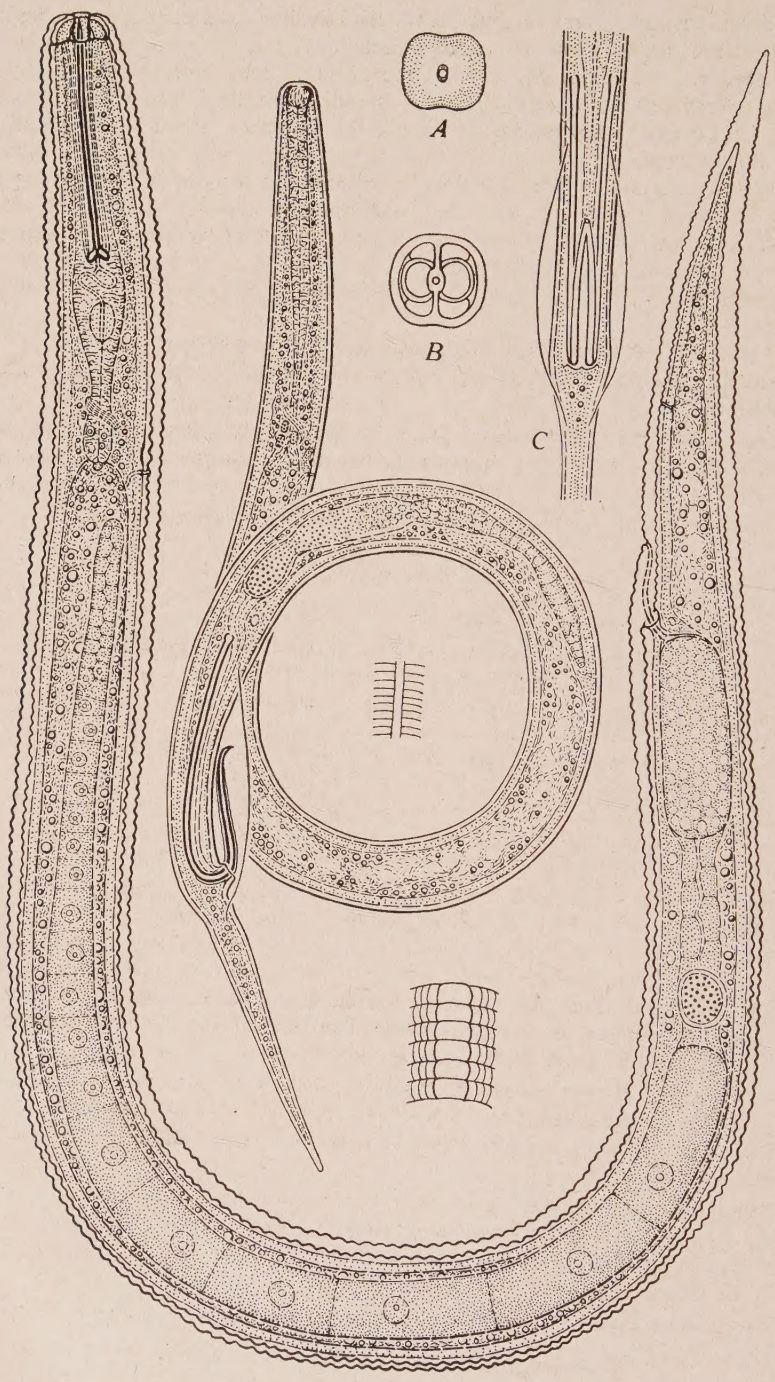

Fig. 3.—*Hemicycliophora penetrans* n. sp. A.—Labial disc of female. B.—Basal pattern of cephalic framework. C.—Male tail, ventral.

Larval cuticle loosely surrounding the slender, slightly arcuate body which is marked by about 250 striae. Details of lateral fields not observed. Neck tapering rapidly to the narrow, round lip region. Posteriorly the body tapers rather uniformly from vulva to terminus. Spear extending through about 30 annules, its basal knobs projecting slightly posteriad. Nerve ring surrounding middle of isthmus. Excretory pore about one body width posterior to esophagus base. Hemizonid not seen. Intestinal cells vacuolated, containing only a few fine granules. Ovary outstretched, about half as long as body. Uterus without spermatheca. Vulva a depressed slit. Anus obscure, probably about 18 annules posterior to vulva. Due to preservation in hot corrosive sublimate fixative, some of the details recorded may not be exactly correct.

DIAGNOSIS.—*Hemicycliophora arcuata* is distinctive because of the slender, slightly arcuate body, narrow lip region and uniformly tapering posterior portion.

TYPE LOCALITY.—Soil about roots of plants imported from Rio de Janiero, Brazil, 1918. Three specimens mounted in balsam under the direction of N. A. Cobb.

TYPE SPECIMEN.—Female, specimen No. 40, slide *Tripyla* 7, NS-U.

Hemicycliophora conida n. sp. (Fig. 5)

Female: 0.8 mm; a = 20; b = 5.4; V — $^{50}_{88}$

Body marked by about 200 annules. Larval cuticle clinging closely to body. Posterior to the vulva very few annules remain on the exterior of the larval cuticle, while on the interior the cuticle is distinctly annulated. Lateral fields marked by 2 parallel lines; adjacent to these lines each annule may have 2 ovate shadowy markings, which are not visible on all specimens. Lip region somewhat truncate with labial disc about one-third as wide as head. Two annules mark the larval cuticle over the lips. Spear extending through about 20 annules. Basal bulb of esophagus slightly clavate. Nerve ring encircling the narrow isthmus near its middle. Excretory pore near beginning of intestine. Hemizonid not observed. Intestinal cells vacuolated, containing a very few small, colorless granules. An egg was 30 μ x 80 μ. Uterus without spermatheca. Vulva located at a ventral contraction of the body with slightly protuberant anterior lip. Anus not observed. Body sometimes uniformly conoid from the vulva to the small rounded terminus, but more often slightly convex or concave-conoid. The tails of the Netherlands specimens are more like those figured for *H. similis*.

DIAGNOSIS.—*Hemicycliophora conida* is distinctive because of the conoid shape of the posterior portion of the body, the two lines marking the lateral field, and the pairs of shadowy ovate bodies on each annule adjacent to the lateral field.

TYPE LOCALITY.—Sugar beet field near Ballyculane, Ireland, 22 specimens; also from sugar beet field near Castle Gregory and Coulmachery, Ireland. Seven specimens from a cultivated field, Gorée, Netherlands, collected by J. W. Seinhorst.

TYPE SPECIMEN.—Female No. 5, slide *Hemicycliophora* 9, NS-U.

Hemicycliophora uniformis n. sp. (Fig. 6)

Female: 0.9 mm; a = 20; b = 4.9; V — $^{35}_{83}$

Larval cuticle clinging rather closely throughout the length of the body.

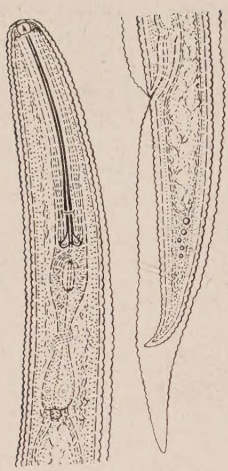

Fig. 4
Hemicycliophora arcuata n. sp.

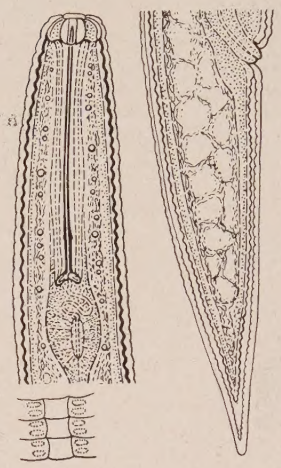

Fig. 5
Hemicycliophora conida n. sp.

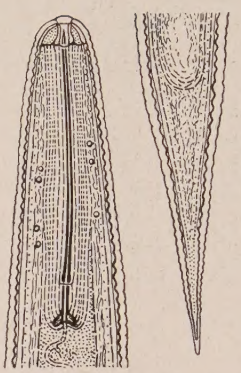

Fig. 6
Hemicycliophora uniformis n. sp.

Cuticle marked by 276-303 striae, without longitudinal markings or shadow-like patterns on the lateral fields. Neck tapering uniformly from opposite the median bulb to the rounded lip region. Labial disc slightly elevated. Spear about one-half as long as vulva-terminus distance. Knobs of spear projecting slightly backward. Median esophageal bulb almost as wide as body cavity; basal bulb distinctly clavate. Excretory pore almost directly opposite anterior end of intestine. Hemizonid not seen. Intestine vacuolated, containing only a few granules. Vulva continuous with body contour except for the elevated lips. Body ending in a uniformly conoid, tapering tail with finely rounded terminus.

DIAGNOSIS.—*Hemicycliophora* with the above general measurements and description. Most closely related to *H. parvana* and *H. conida* from which it differs in the narrowed, conoid lip region and the ratios of the spear lengths to vulva-terminus distances.

TYPE LOCALITY.—Soil, Rockcliffe Park, Ottawa, Ontario, Canada, 21 females and three young collected by S. A. Sher.

TYPE SPECIMEN.—Female No. 6, slide *Hemicycliophora* 1, collection of S. A. Sher.

Hemicycliophora gracilis n. sp. (Fig. 7)

Female: 1.3-1.7 mm; a = 27; b = 6.1; V — $^{40}_{81}$

Body with 341-390 annules. Larval cuticle fitting loosely about body. Lateral fields marked by 2 longitudinal lines which are obscure on many specimens. Lip region rounded, with 2 large annules. Spear length equal to about 30 of the neck annules. Knobs of spear extending somewhat backward. Vulva-terminus distance two and one-half times spear length. Basal bulb of esophagus clavate. Excretory pore about one body width posterior to base of esophagus, its tube leading back about 5 body widths to join the renette gland on the right side of the body. Hemizonid not observed. Intestinal cells somewhat vacuolated, containing a few scattered granules. Ovary sometimes reaching as far as the median esophageal bulb. Uterus without sperma-

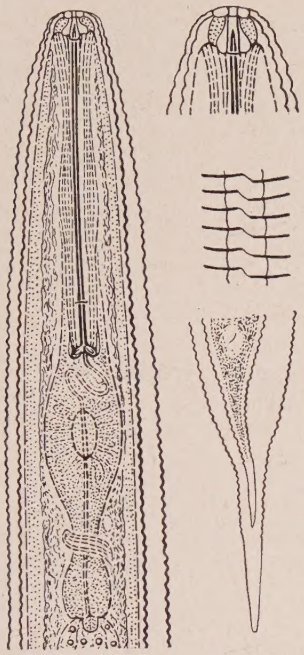

Fig. 7.—*Hemicycliophora gracilis* n. sp.

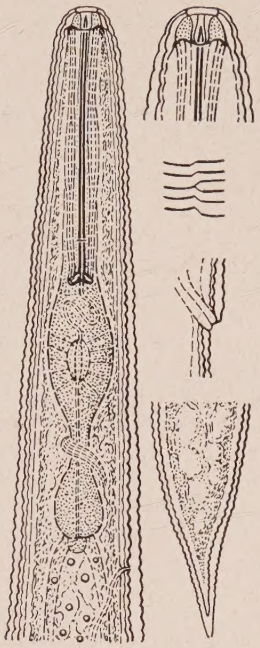

Fig. 8.—*Hemicycliophora similis* n. sp.

theca. Vulva 70 to 80 annules from terminus. Body slightly contracted posterior to vulva. Eggs averaging about 30 x 100 μ. Anus very obscure, 20-30 annules behind vulva. Tail convex-conoid, then spicate. Annules absent on terminal third of spicate portion.

DIAGNOSIS.—*Hemicycliophora* with the above general description. Distinctive because of the 2 lines on the lateral field, the rounded lip region and the convex-conoid tail ending in a spicate terminus.

TYPE LOCALITY.—Soil from stream bank, Broad Run, Virginia. Also collected from a peach orchard near South Haven, Michigan; pine barrens, Monmouth County, New Jersey; grass roots near Raleigh, North Carolina; and about gladiolus corms, Grants Pass, Oregon. The Oregon specimens were collected by Harold J. Jensen.

TYPE SPECIMEN.—Female No. 6, slide *Hemicycliophora* 1, NS-U.

Hemicycliophora similis n. sp. (Fig. 8)

Female: 1.1 mm; a = 23; b = 5.6; V — $^{51}_{79}$

Cuticle marked by about 290-307 annules. Larval cuticle fitting snugly about body. Lateral fields simple without longitudinal markings. Lip region bearing 2 annules. Spear, with rounded basal knobs, extending through 22-34 annules, its length one-half the vulva-terminus distance. Basal bulb of esophagus pyriform, about one-third as wide as body. Nerve ring encircling the narrow isthmus near its middle. Excretory pore opposite anterior end of intestine. Hemizonid not observed. Intestinal cells vacuolated containing only a few scattered granules. Ovary outstretched, generally about one-half as long as body. Uterus without spermatheca. Vulva located at a ventral

contraction 48-66 annules from the terminus. Anus exceedingly obscure about 20 annules behind vulva. Tail convex-conoid.

DIAGNOSIS.—*Hemicycliophora similis* is practically identical to the females of *H. typica,* and differs only in the fact that the species is monosexual and that there is no spermatheca in the uterus.

TYPE LOCALITY.—Grass sod in mountain meadow, Blacks Fork, Uintah Mountains, Utah. Also from alfalfa field, Yerington, Nevada; peach orchard, California; greenhouse soil, Denver, Colorado. Collected about grass roots, Quebec, Canada by S. A. Sher.

TYPE SPECIMEN.—Female No. 3, slide *Hemicycliophora,* 2, NS-U.

Hemicycliophora typica de Man, 1921 (Fig. 9)

Female: 1.2 mm; a = 20; b = 6.0; V — $^{52}_{84}$

Body marked by 256-280 annules. Larval cuticle fitting rather closely. Lateral fields simple without longitudinal markings. Lip region rounded with 2 annules in last larval cuticle, but none on lips proper. Spear extending through 19-26 annules, the basal knobs projecting slightly backward. Basal bulb of esophagus slightly clavate. Excretory pore opposite beginning of intestine. Hemizonid small but distinct, adjacent to excretory pore. Intestinal cells vacuolated, containing small nuclei. Ovary outstreched, about one-half as long as body. Uterus with prominent spermatheca. Vulva with protuberant posterior lip, located at a ventral contraction of the body. Anus very obscure, 14-17 annules posterior to vulva. Tail at first convex-conoid, then elongate conoid to the small sharp terminus.

Male: 1.0 mm; a = 34; b = 6.2; c = 5.1; T — 42

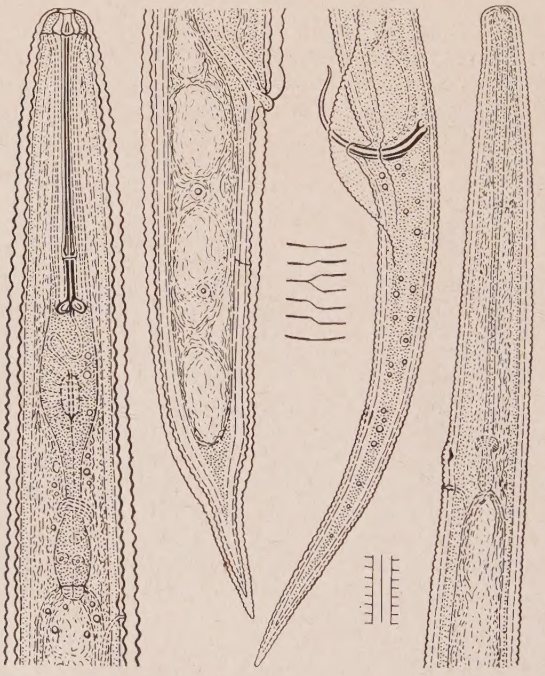

Fig. 9.—*Hemicycliophora typica* deMan

Cuticle marked by fine but distinct striae. Lateral fields with 3 lines forming 2 bands which extend from near the head to near the middle of tail. Lip region slightly expanded. Spear absent. Pharynx sometimes appearing as an open chamber with slightly sclerotized walls. Esophagus a slender tube with nerve ring near its base. Excretory pore opposite base of esophagus. Hemizonid very distinct, slightly anterior to excretory pore. Intestinal cells vacuolated, with scattered granules. Testis outstretched, slightly less than one-half body length. Spicula semi-circular, as illustrated. Bursa broad, about 3 times as long as body width. Tail conoid to the small pointed terminus.

DIAGNOSIS.—*Hemicycliophora typica* is distinctive because of the tail form, the simple lateral fields and the semi-circular spicula.

TYPE LOCALITY.—Cultivated field, Zeeland, Holland.

NEOTYPE.—Female No. 2, slide *Hemicycliophora* 6.

NEOALLOTYPE.—Male No. 1, slide *Hemicycliophora* 6 a, University of California, Berkeley.

Hemicycliophora brevis n. sp. (Fig. 10

Female: 1.0 mm; a = 19; b = 5.5; V = $^{70}_{90}$

Body marked by 250 coarse annules which are not interrupted by longitudinal lateral lines. Larval cuticle fitting loosely about body. Neck tapering slightly to the broad, truncated lip region. Spear extending through only 23 annules, its length greater than the vulva-terminus distance. Esophagus about three-fourths as long as spear with clavate basal bulb. Excretory pore about opposite base of median esophageal bulb. Hemizonid not observed. Intestinal cells vacuolated, containing a few scattered granules. Body constricted behind the vulva which has prominent, protruding labia. Body tapering gradually from the vulva to the latitude of the end of the intestine, then narrowing rapidly through the conoid tail which ends in a blunt terminus.

DIAGNOSIS.—*Hemicycliophora brevis* is immediately distinguished by the unusually short vulva-terminus distance and the more anterior location of the excretory pore.

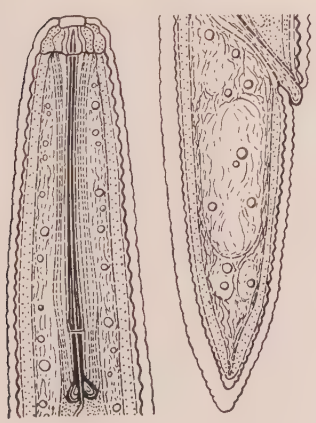

Fig. 10
Hemicycliophora brevis n. sp.

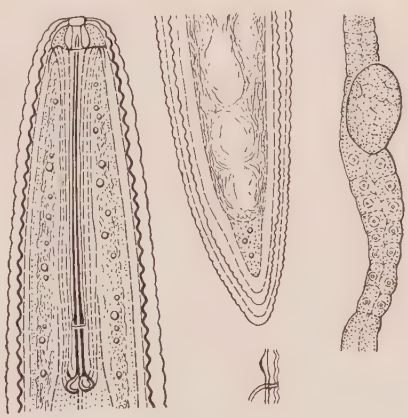

Fig. 11
Hemicycliophora obesa n. sp. Head, tail, excretory pore and hemizonid, and uterus with spermatheca.

TYPE LOCALITY.—Inverness, California, about roots of bay tree. A single female collected by M. W. Allen.

TYPE SPECIMEN.—Female, slide *Hemicycliophora* 1, University of California, Berkeley.

Hemicycliophora obesa n. sp. (Fig. 11)

Female: 1.1 mm; a = 14; b = 5.0; V — $^{42}_{84}$

Larval cuticle fitting loosely about the body which is marked by 259-279 annules. Lateral fields without longitudinal markings. Lip region rounded, marked by 2 annules. Spear two-thirds as long as vulva-terminus distance, extending through 23 annules. Excretory pore slightly posterior to base of esophagus. Hemizonid one annule anterior to excretory pore. Intestinal cells vacuolated, each with a small nucleus. Ovary about one-half as long as body. Uterus with a well developed spermatheca, but spermatozoa were not present and males are unknown. Vulva depressed, located at a slight ventral contraction of the body. Anus not seen. Tail somewhat convex-conoid to the blunt terminus.

DIAGNOSIS.—*Hemicycliophora obesa* is distinctive because of its greater body width, form of the tail (as shown), and well developed spermatheca.

TYPE LOCALITY.—Sixteen specimens from soil about alpine plants, Brighton, Utah.

TYPE SPECIMEN.—Female No. 8, slide *Hemicycliophora* 10, NS-U.

Hemicycliophora aberrans n. sp. (Fig. 12)

Female: 1.0 mm; a = 18; b = 5; V — $^{52}_{88}$

Body generally cylindroid with a very distinct ventral contraction at the vulva. Lateral cuticle clinging so closely to the body that almost all traces of annules have disappeared. Lateral fields simple without longitudinal lines. Lip region with elevated labial disc. One broad annule occupies almost the entire lip region. Spear with very strongly developed basal knobs, extending through about 31 annules, longer than the vulva-terminus distance. Basal bulb of esophagus slightly clavate, the nerve ring encircling its anterior end. Hemizonid not observed. Intestinal cells vacuolated, with very few small

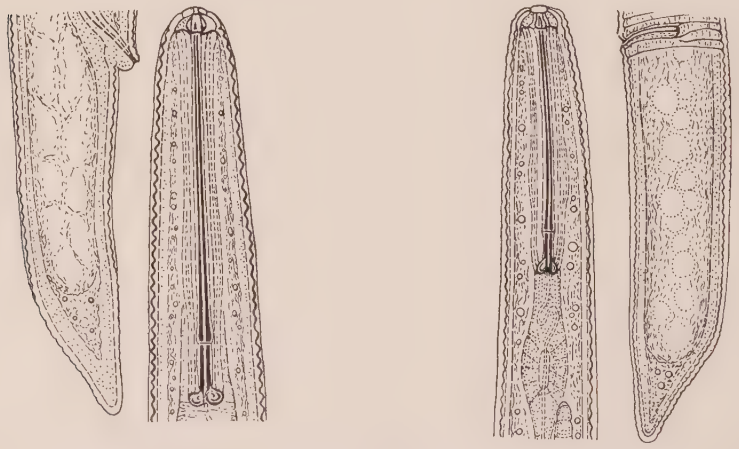

Fig. 12.—*Hemicycliophora aberrans*, n. sp. Fig. 13.—*Hemicycliophora striatula* n. sp.

colorless granules. Ovary outstretched, about one-half as long as body. Uterus without spermatheca. Vulva with protuberant lips. Anus not observed. Tail irregular conoid to the small rounded terminus.

DIAGNOSIS.—*Hemicycliophora aberrans* is distinctive because of the tightly stretched larval cuticle, protuberant lip of vulva, prominent ventral contraction of the body at vulva and irregular-conoid tail. The tail form is not constant, some specimens being slightly longer or shorter than the one figured.

TYPE LOCALITY.—Three females from stream bank soil, Little Cottonwood Canyon, near Salt Lake City, Utah.

TYPE SPECIMEN.—Female No. 1, slide *Hemicycliophora* 6, NS-U.

Hemicycliophora striatula n. sp. (Fig. 13)

Female: 0.8 mm; a = 19; b = 5; V — $^{70}_{90}$

Neck tapering but little to the truncated lip region. Larval cuticle closely clinging to body. Annules of larval cuticle visible near head and tail, but on remainder of body the cuticle is stretched until the annules lose their identity. Lateral fields plain, without markings. Spear extending through 23 annules, the basal knobs projecting slightly backward. Vulva-anus distance one and one-half times spear length, marked by 56 annules. Basal esophageal bulb clavate. Intestine vacuolated, with very few granules. Vulva almost continuous with body contour. Ovary extending forward to base of median esophageal bulb. Excretory pore near anterior end of intestine. Hemizonid not observed.

DIAGNOSIS.—*Hemicycliophora* with the above measurements and general description. Differs from *H. aberrans,* apparently its closest relative, in the shorter spear, truncated head and number of annules in the vulva-anus region.

TYPE LOCALITY.—One female and seven young from soil about roots of manzanita, *Arctostaphylos* sp., Napa County, California. Collected by M. W. Allen.

TYPE SPECIMEN.—Female on slide *Hemicycliophora* 6, University of California, Berkeley.

Hemicycliophora obtusa n. sp. (Fig. 14)

Female: 0.8 mm; a = 20; b = 5; V — $^{46}_{87}$

Cuticle marked by 235 and 256 annules on the 2 specimens examined, the annules extending almost to terminus. Larval cuticle clinging closely to body, smooth and rounded on tail. Lateral fields plain without longitudinal markings. Lip region marked by two annules. About 4 annules of the larval cuticle cover the lip region. Spear slightly longer than vulva-terminus distance, with strongly developed, rounded basal knobs. Basal bulb of esophagus well developed, about one-half as wide as body. Nerve ring encircling anterior end of basal bulb. Excretory pore opposite beginning of intestine. Hemizonid not observed. Intestinal cells vacuolated, containing a few fine, scattered granules. Ovary outstretched, about one-half as long as body. Uterus without spermatheca. Vulva located at a ventral contraction 33 and 38 annules from the terminus on the 2 specimens. Anus obscure, about 20 annules posterior to vulva. Tail almost hemispheroid.

DIAGNOSIS.—*Hemicycliophora obtusa* is distinctive among the round tailed species because of the rounded lip region with its 2 annules, the closely clinging larval cuticle which is smooth over the blunt rounded tail, and the ventral contraction of the body at the vulva.

TYPE LOCALITY.—Sugar beet field near Lewiston, Utah.
TYPE SPECIMEN.—Female No. 1, slide *Hemicycliophora* 3, NS-U.

Hemicycliophora nana n. sp. (Fig. 15)

Female: 0.7 mm; a = 18; b = 4.6; V — 87

Body almost cylindrical throughout its length, tapering very slightly posterior to the vulva and ending in a hemispheroid tail. Annules 222 and 236 on the 2 specimens collected. The last 2 larval cuticles are retained and fit very closely about the body. On the outer cuticle the annules disappear over the rounded tail. Lateral fields plain without longitudinal markings. Lip region truncated, from a lateral view appearing to be divided into 3 almost equal portions. Spear extending through about 33 annules, its basal knobs projecting slightly backward. Basal bulb of esophagus somewhat clavate. Excretory pore just anterior to the base of the esophagus. Hemizonid not observed. Intestinal cells large, vacuolated, containing a very few small granules. Details of ovary not observed because of dense body tissues. Vulva slightly elevated above body contour. Anus not observed.

DIAGNOSIS.—*Hemicycliophora nana* is distinctive because of the cylindroid body, the fact that the 2 last larval cuticles are retained and that there is no ventral contraction of the body at the vulva.

TYPE LOCALITY.—Red Moutain north of Vernal, Utah, soil about alpine plants at an elevation of about 8,500 feet.
TYPE SPECIMEN.—Female No. 2, slide *Hemicycliophora* 6, NS-U.

Hemicycliophora rotundicauda n. sp. (Fig. 16)

Female: 1.2 mm; a = 23; b = 5.1; V — $^{49}_{87}$

Body marked by 255-275 annules. Larval cuticle fitting closely about body. Lip region rounded, bearing two broad annules. Tail hemispherical, without annules near the terminus. Spear about two-thirds as long as vulva-terminus distance, slightly arcuate, extending through 20 annules, its knobs rounded and not projecting backward. Median bulb of esophagus almost as wide as body cavity; basal bulb clavate, twice as wide as isthmus. Nerve ring crossing isthmus at beginning of basal bulb. Intestine vacuolated, with only a few small granules. Body ventrally contracted at vulva. First stage larva

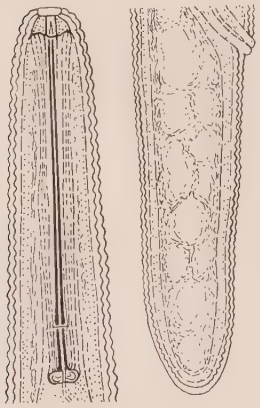

Fig. 14.—*Hemicycliophora obtusa* n. sp.

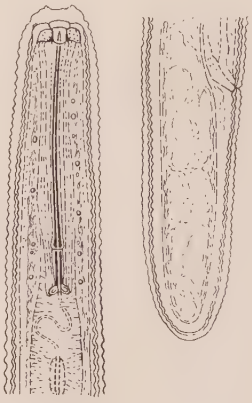

Fig. 15.—*Hemicycliophora nana* n. sp.

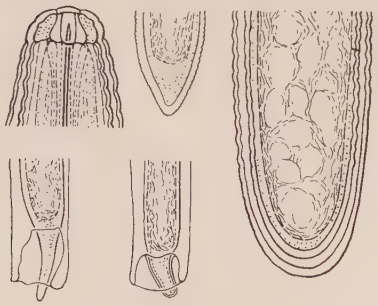

Fig. 16.—*Hemicycliophora rotundicauda* n. sp. Tails of first, second and third larval stages and head and tail of adult female.

with an elongated, convex-conoid tail which becomes shorter and the terminus more rounded with each moult, as illustrated.

DIAGNOSIS.—*Hemicycliophora* with the above measurements and general description. Most clearly related to *H. obtusa* from which it differs in its larger size and in the form of the lip region.

TYPE LOCALITY.—Soil about base of a conifer, Echo Lake, Mount Evans, Colorado. Collected by D. J. Raski.

TYPE SPECIMEN.—Female No. 2, slide *Hemicycliophora* 2, University of California, Berkeley.

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Xiphinema krugi n. sp. (Nematoda, Dorylaimidae) from Brazil with a key to the species of *Xiphinema*

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A new species of *Xiphinema* was collected by the writer in the State of São Paulo, Brazil. This new *Xiphinema,* here described as *X. krugi* n. sp., represents the third species of the genus found in that state. The previously described forms are *X. brasiliense* Lordello, 1951, and *X. campinense* Lordello, 1951.

Female: 2.12-2.22 mm. a = 37.9-43.8; b = 5.2-5.6; c = 66.3-69.6; V = 33.4-34.2.

Body practically cylindrical, tapering only slightly near the extremities. Head contour absolutely smooth, lips amalgamated; lip region almost continuous with the neck contour, set off only by a very weak depression; labial papillae extremely small, one circle having been observed.

Cuticle showing fine transverse striations. Lateral field narrow on the neck, but gradually becoming wider; at level of the vulva, they are one-sixth to one seventh as wide as body. Tail short, subconoid.

Length of spear ranging from 116.0 to 120.0 μ and length of spear extension from 68.0 to 72.0 microns, making a total of 184.0 to 192.0 microns. The spear is slightly ventrally arcuate. The guiding ring of spear sometimes seems to consist of two parts, one of which is always easily seen. Anterior portion of oesophagus a slender and flexible tube, often somewhat convoluted when the stylet is retracted; posterior portion abruptly expanding, forming an elongate basal bulb, which measures 96.0 × 24.0 microns. Lumen of the bulb very conspicuous. The nucleus of the dorsal oesophageal gland is located at the extreme anterior end of the basal bulb, but is often obscure. Nuclei of the anterior submedian pair of oesophageal glands are located at about the middle of the bulb, but the nuclei of the posterior submedian glands were not located.

Cardia conoid, developed to different degrees in the various individuals studied. In a few females, a structure on the level of the cardia was observed. Such a structure appears as rather thick lines directed backward, for which no satisfactory explanation has been found. Sometimes they seem to be crystalized matter.

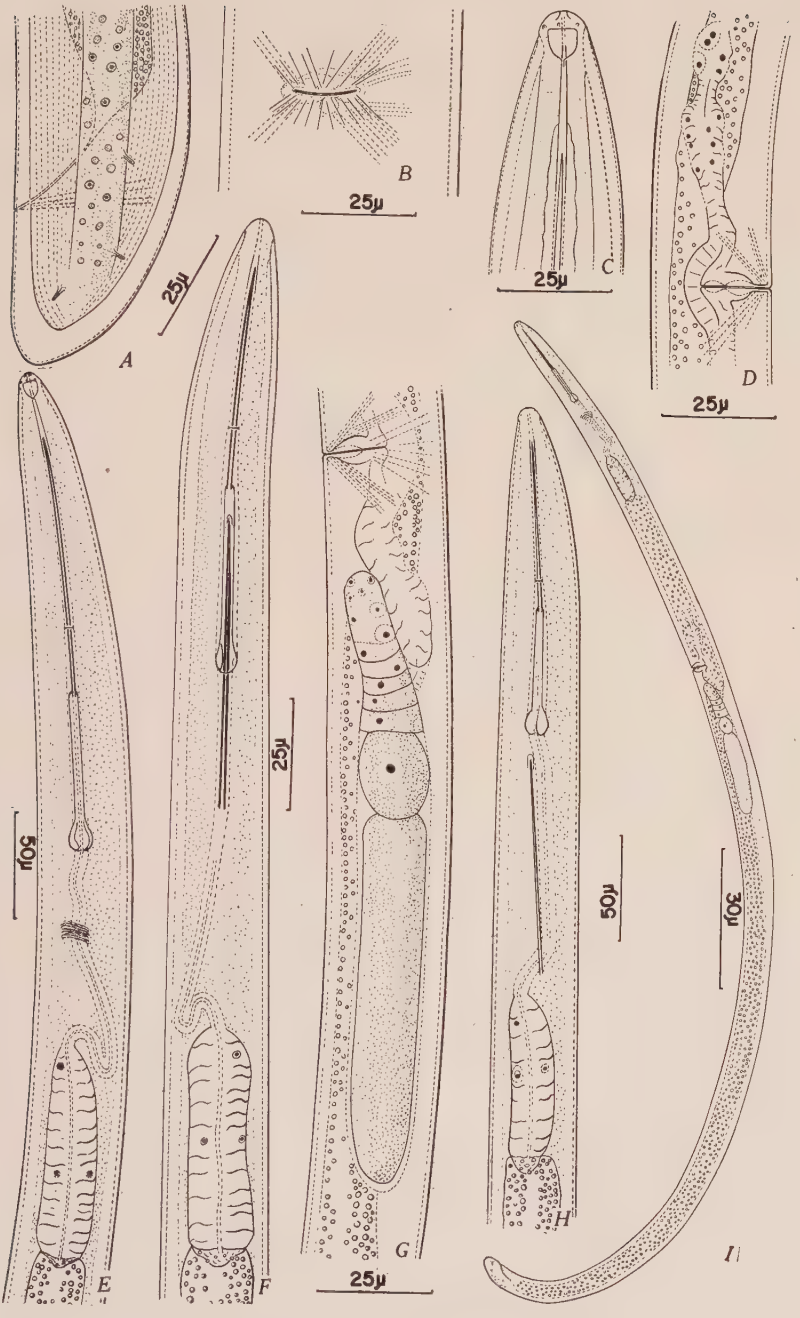

Fig. 1.—*Xiphinema krugi* n. sp. A. Posterior end of female. B. Ventral view of the vulva showing three series of muscles. C. Head of the female. D. Anterior ovary. E. Oesophageal region of the female. F. Oesophageal region of a larva showing new spear passing through lumen of the spear extension. G. Posterior ovary. H. Oesophageal region of a larva with a new spear. I. Adult female.

Intestine 6 cells in circumference; usually the intestinal cells appear full of dark granules. A few cross sections of the intestine were made according to the techniques given by Buhrer (1949). The intestinal cells are rather high and the lumen narrow, indicating extra-oral digestion and, of course, parasitic habits. No differentiation between pre-rectum and rectum was noticed. The anus is located in a slight depression.

Three pairs of prominent caudal papillae are present, which are arranged as illustrated (fig. 1, A). However, in one individual, the two posterior pairs apeared to be closer together than shown in the figure.

The amphids are rather large (more than half as wide as lip region), and a slender obscure tube can sometimes be seen posterior to the amphids.

Ovaries two, the posterior one being normal and well developed, and the anterior one being much reduced and very obscure. In some specimens, it seemed to be outstretched but in other individuals a short reflexion was noticed. The writer intends to make further observations on this point, as soon as new material has been collected. The present observations however led to the conclusion that it is not a functional ovary, but rather in process of disappearance. According to this, *X. krugi* n. sp. represents an intermediate form between the true amphidelphic and the monodelphic species.

As it was not possible to find a single specimen showing the two ovaries well, even among those stained by acid carmine, it was necessary to make two drawings from two different individuals. The nuclei represented in fig. 1, D (anterior ovary) are not nuclei of eggs, but of the cells of the wall of an oviduct, like those in the uterus. The oviduct is set off from the uterus by a rather deep depression.

Vulva a transverse slit two-fifths as wide as the body. Vagina extends half-way across the body. There are well developed muscles connected with the vulva and vagina, whose arrangement is rather interesting. Three series of muscles could be seen through the study of a ventral view of the region, as in fig. 1, B. The innermost and the middle series are stronger than the uppermost one and are connected with the vagina in order to open it when an egg is being deposited. The uppermost series is attached to the vulva.

As previously stated, the posterior ovary is normal. The reflexed part sometimes reaches the level of the vulva. The oocytes are arranged in a single line, except for the end portion of the ovary, in which a multiple line of nuclei of oocytes could be seen. Only one very large egg, which measures 284.0 × 36.0 microns, is present in the uterus at a time. Therefore, the diameter of the egg represents about 75.0% of the body width.

Observations about the larvae—The tail of the larvae is more conoid and elongate than that of the adult and the caudal papillae are less prominent.

Among the larvae collected, there were a few having a new spear (odontostylet) in different positions in the anterior portion of the oesophagus (fig. 1, H). The base of the new spear sometimes shows three points which of course are related with the connection with the spear extension. As far as we know, the spear is formed by a cell or group of cells located near the bulb of the oesophagus, being translocated in order to take the position of the former, which is shed with the cuticle when the specimen is moulting.

The most interesting phase of this fascinating process that we have found in this species, shows the new spear going through the narrow lumen of the spear extension (fig. 1, F). The time required for the translocation of the new spear across the canal of the extension is unknown. But, during this period, in spite of being unable to feed, the larvae seem to be active, as we

TABLE 1.—Measurements in micra of 4 larvae of *Xiphinema krugi* n. sp.

Measurement	Larvae			
	1	2	3	4
Length	836.0	942.0	1,412.0	1,470.0
Width	22.0	28.0	40.0	32.0
Length of oseoph.	232.0	264.0	364.0	298.0
Stylet	88.0	108.0	144.0	126.0
Spear	48.0	64.0	84.0	72.0
New spear	66.0	84.0	112.0	90.0
Tail	24.0	36.0	36.0	40.0

obtained one by the Baermann funnel technique. In addition, in spite of already having about half of the new spear inside the extension, the specimen studied does not show any other indication of the moulting process, the cuticle being still firmly attached to the body. This shows that the phenomena are not synchronic. As expected, the new spear is always longer than the one to be replaced. The spear extension also grows during the moulting processes, since that of the adult females is always longer than the ones of the different larvae measured. The dimensions of four larvae, which represent at least two stages of the cycle, are given in table 1. The first larva is the smallest one found in the population studied.

MALES unknown.

HABITAT: A few females and larvae were collected from soil around several native forest trees, in a wood located at Piracicaba, State of S. Paulo, Brazil. The smooth head contour and the long stylet are considered good indications of parasitism on roots. The specific name is given in honor of Dr. C. A. Krug, Director of the Instituto Agronômico, Campinas, Brazil.

DIAGNOSIS: *X. krugi* was compared with the species monographed by Thorne (1939) as well as with those described by Schuurmans Stekhoven & Teunissen (1938), Loos (1949), Schuurmans Stekhoven (1951), Thorne & Allen (1950), Meyl (1953) and the writer (1951, 1951a, 1953).

The species most closely resembling *X. krugi* n. sp. is *X. ensiculiferum* (Cobb, 1893) Thorne, 1937, especially the population studied by Loos (1949). *X. ensiculiferum* was described by Cobb in 1893 as *Tylencholaimus ensiculiferus,* from a collection obtained from the soil about roots of banana plants in Fiji. In 1949, Loos found the species in Ceylon and published a redescription, including a study of the male.

The differences in the shape of the tail (subconoid in *X. krugi* and rounded in *X. ensiculiferum*) and in the organization of the reproductive apparatus (*X. krugi* has two ovaries and *X. ensiculiferum* only one) justify the establishment of the new species.

Key to the species of the genus *Xiphinema* Cobb, 1913

As several new forms of "dagger nematodes" have been described since the publication of Thorne's monograph (1939), the writer organized the following key for the separation of the species known to date. Only female characters were used in the key, as the *Xiphinema* males are frequently rare and unknown in some species. Unfortunately, it was not possible to place in the key the species described by Schuurmans Stekhoven in 1951 (*X. brevicaudatum, X. effilatum* and *X. digiticaudatum*) as they were based on larvae.

In previous publications, the writer emended the names of three species of the genus, because they were established either by a printing error or a *lapsus calami.* The writer's procedure was based on the 19th article of the Interna-

tional Rules of Zoological Nomenclature (*apud* Amaral, 1950). These three forms are: *X. grande* Steiner, 1914, *X. pratense* Loos, 1949 and *X. insigne* Loos, 1949, formerly referred to as *X. grandis, X. pratensis* and *X. insignis.*

1. Ovary one. 2
   Ovaries two. 5
2. Tail rounded and short. ..... *X. ensiculiferum* (Cobb, 1893) Thorne, 1937
   Tail elongated, conoid or digitate. 3
3. Two pairs of caudal papillae present. 4
   Three pairs of caudal papillae present. ..... *X. radicicola* Goodey, 1936
4. Tail elongate, amphids narrow and long. ..... *X. chambersi* Thorne, 1939
   Tail distinctly digitate, amphids wide and short. ..... *X. brasiliense* Lordello, 1951
5. Tail short and rounded 6
   Tail elongate, conoid, subconoid or digitate. 9
6. Lip region set off by constriction. 7
   Lip region continuous. 8
7. Small species (1.616 mm), V = 40.0%, oesophagus short (b = 6) ..... *X. grande* Steiner, 1914
   Large species (4.0 mm), V = 60.0%, oesophagus very long (b = 2) ..... *X. makrodorum* (Vanha, 1893) Thorne, 1939
8. Small species (0.8 mm) ..... *X. obtusum* Cobb, 1939
   Large species (4.1 mm) ..... *X. rotundatum* Sch. Stekhoven & Teunissen, 1938
9. Lip region expanded. ..... *X. lineum* (Grube, 1849) Thorne, 1937
   Lip region not expanded. 10
10. Head truncate. ..... *X. truncatum* Thorne, 1939
    Head more or less rounded, not truncate. 11
11. Length 3.4-4.0 mm or much larger (8.94 mm). 12
    Length 2.60 mm or less. 14
12. V = 38.0% ..... *X. index* Thorne & Allen, 1950
    V = 47.8-48.0% 13
13. Length 8.94 mm. ..... *X. cylindricaudatum* Sch. Stekhoven & Teunissen, 1938
    Length 4.0 mm. ..... *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939
14. V = 51.0-54.0% ..... *X. americanum* Cobb, 1913
    V = 29.8-47.0% 15
15. Tail subconoid or digitate, not ventrally arcuate. 16
    Tail distinctly elongated, more or less deeply ventrally arcuated. 17
16. Tail distinctly digitate (55.5 μ long), V = 39.4%. ..... *X. mammillatum* Sch. Stekhoven & Teunissen, 1938
    Tail not digitate, subconoid (32 μ long), V = 33.4-34.2%. ..... *X. krugi* n. sp.
17. Four pairs of caudal papillae present. ..... *X. campinense* Lordello, 1951
    More than 4 pairs of caudal papillae present. 18
18. Combined length of spear and spear extension shorter (123.4 μ). ..... *X. italiae* Meyl, 1953
    Combined length of spear and spear extension longer (142.0-158.0 μ). 19
19. Six pairs of caudal papillae present, spear apparently consisting of two parts. ..... *X. elongatum* Sch. Stekhoven & Teunissen, 1938
    Seven pairs of caudal papillae present, spear consisting of only one part, as is usual. 20

20. Tail long (77.0-102.0 μ), V = 29.8-31.6%, with a cuticular triangular-shaped structure in the anterior slender part of the oesophagus. ——— ——— *X. insigne* Loos, 1949

Tail short (48.0-57.0 μ), V = 39.0-42.0%, without such a triangular-shaped structure in the anterior portion of the oesophagus. ——— ——— *X. pratense* Loos, 1949

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ACKNOWLEDGEMENTS

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A Comparison of the Thermal Death Time of Two Dissimilar Species of Nematodes: *Panagrellus redivivis* (Linn. 1767) Goodey 1945, and *Meloidogyne incognita* var. *acrita*, Chitwood, 1949

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In recent years the free-living saprophytic nematodes have become popular test organisms for the routine screening of chemicals for use as agricultural nematocides and anthelmintics. Certain species of *Panagrellus,* being widespread in distribution and easily cultured on starchy or carbohydrate media are admirably suited to screening procedures. *Panagrellus redivivis,* (Linn., 1767) Goodey, 1945, is actively motile and since the loss of motility of individual worms can be easily translated to a quantitative response to chemical treatment, it is usually the organism of choice. Tarjan (1953) has reported on chemical compounds screened against this organism. However, some physiological similarities must exist between the test nematode and the plant and animal parasites or the test organism must be rejected as unsatisfactory.

The lack of comparative physiological data which would link the "in vitro" activity of *P. redivivis* to the "in vivo" activity of the plant parasites has led to these studies comparing the thermal death times of two dissimilar species of nematodes: *P. redivivis* and *Meloidogyne incognita* var. *acrita,* Chitwood, 1949. Treffitt and Hurst (1935) after investigating hot water treatments on *Heterodera schachtii,* Schmidt, 1871, concluded that 130° F. (54° C.) was the lowest temperature at which all encysted larvae were killed after a five minute exposure. Mai and Lautz (1953) reported that the free larvae of *Heterodera rostochiensis,* Wollenweber, 1923, could not survive a temperature of 125°F. (52°C.) for five minutes and the encysted larvae were killed after five minutes of exposure to 131°F. (55°C.) Cairns (1953) in working with *Ditylenechus* sp. found that the death of nematodes in an anabiotic state show temperature-time gradients which varied with the moisture level. The lower the level the wider the gradient and the higher the resistance. The minimum, the optimum, and the maximum temperatures for activity have been determined for many enzyme systems. Most animal enzymes have an optimum near 40°C. and are quickly inactivated by heating above 50°C. Thermal death after short exposure to temperature near or above 50°C. is not surprising from the standpoint of enzyme inactivation. It is probable that the difference in thermal death points between the more or less protected encysted larvae and the unprotected free larvae of *H. rostochiensis* is caused by thermal insulation rather than by basic physiological differences.

In the work reported here, the materials used were nematodes from cultures containing all stages of development of *P. redivivis,* and egg masses of the root-knot nematode, *M. incognita* var. *acrita.* Since *P. redivivis* is viviparous, the female uterus contains eggs in all stages of development as well as larvae. The eggs of the root-knot nematode are deposited in a very early stage of development and egg masses contain eggs in the single cell stage, larvae which have hatched from the eggs, and all intermediate stages. These are embedded in a protective jelly-like substance. In an endeavor to eliminate the possi-

bility of thermal insulation of the body of the female *P. redivivis* and that of covering of the egg mass, of *M. incognita* var. *acrita,* the experiments were conducted at lower temperature over more extended periods of time.

METHODS

Nematodes of the species *P. redivivis* were maintained in a culture medium of oatmeal, prepared by boiling 10 grams of "Quick Oats" in 100 ml. of tap water. Worms for experimentation were selected from young and vigorous cultures in the logarithmic phase of population expansion. The nematodes were separated from the oatmeal medium by passage through a Baermann funnel. Ten ml. of a nematode suspension containing approximately 500 worms were added to each of fourteen 20 x 150 mm. test tubes and then allowed to settle. After the worms had settled to the bottom of the tube, nine ml. of supernatant fluid was drawn off by pipette and discarded. Thus, fourteen tubes were prepared each containing one ml. of water and approximately 500 worms in all stages of development.

Root-knot nematodes, *M. incognita* var. *acrita* were cultured on live tomato roots maintained in greenhouse pot cultures. Live worms and viable eggs were obtained from root tissue by grinding the tissue in a Waring Blender. Coarse particles of root tissue were removed by screening and egg masses were separated from the debris under the dissecting microscope. Two egg masses containing 500-600 eggs each were added to one ml. of water in each of fourteen 20 x 150 mm. test tubes.

Twelve tubes of each species were fitted into a holder and submerged 5 cm. in a thermostatically controlled constant temperature water bath, accurate to ±0.5°C. Two tubes of each species were held at room temperature as controls. The experiments were conducted at water bath temperature of 40°, 41°, 42°, 43°, 44°, 45°, and 46° C. All experiments were replicated four times for each temperature. At one-hour intervals for a period of six hours, two tubes of each species were removed from the water bath and immediately cooled to 20°C. by holding under the tap. Previous experiment had shown that one ml. of water at 20°C. in a 20 x 150 mm. test tube immersed 5 cm. in the water bath at 45°C. will equilibrate with the bath in approximately ten minutes. In all experiments the period of equilibration is included in the first hour of exposure.

Each tube of *Panagrellus* was poured into a sterile petri dish of oatmeal medium. The petri dish cultures of the worms exposed to heat treatment and the controls were incubated at room temperature for fourteen days, and then examined for living worms.

The tubes containing the suspensions of root-knot eggs were used to inoculate the roots of two-week-old tomato plants growing in previously sterilized soil. After twelve weeks the tomato plants were harvested and the roots examined for evidences of root-knot infection.

RESULTS

In these experiments no attempt was made to determine the percent mortality resulting from any temperature treatment, rather the results were considered as an "all or nothing" response to heat of the most resistant forms of each species. Any evidence of living worms in the petri-dish cultures of *Panagrellus* or any microscopic evidence of root-knot infection in any of the inoculated tomatoes was considered a positive result, thus only eggs, larvae, or adults surviving the heat treatment could give a positive result under the conditions of the experiments.

In general, the results of the replications were uniform. However, in the *Panagrellus* experiments there were two exceptions: the treatment at 44°C. gave negative culture for three replicates after five hours exposure, and one replicate gave a negative culture after exposure for three hours. In the treatment at 45°C. three replicates gave negative cultures after an exposure of two hours while the fourth replicate gave negative results after three hours of exposure. The maximum survival time was accepted as the true value. Four results in the *Meloidogyne* experiments were discarded because of the death of the tomato plants.

The thermal death time for each species of nematode is represented graphically in Figure 1 where the period of exposure necessary to kill the organism is plotted against the temperature. The lines representing the thermal death time have different slopes for the two species studied. Although the lines intersect and neither species can survive one hour of exposure to a temperature of 46°C., it is obvious that for temperature lower than 46°C. the

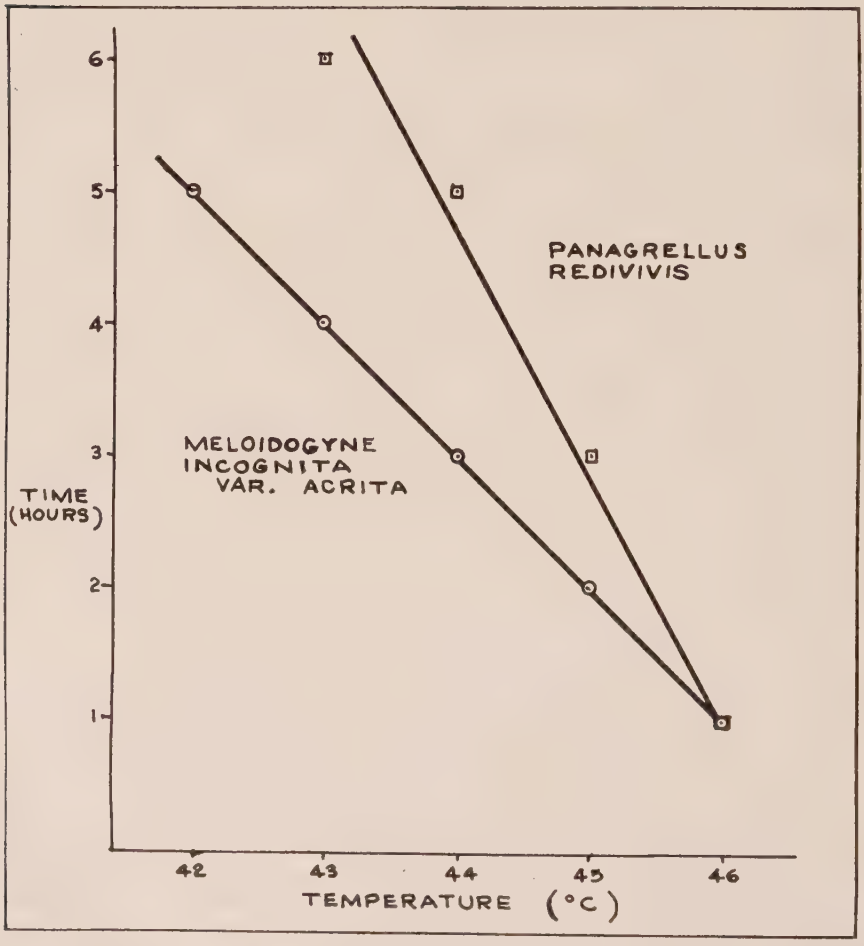

Fig. 1.—Thermal Death Time

Panagrellus species is more heat resistant. Since saprophytes in general show a greater growth temperature range, it is not surprising to find here that the saprophytic *P. redivivis* shows greater heat resistance than the more specialized parasitic *Meloidogyne* species.

From the standpoint of enzyme inactivation the differences in thermal death time exhibited here may represent greater physiological differences between *P. redivivius* and the parasitic nematode species than has been assumed in the past.

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Cyclustra ardeae n. sp. and the status of *Dendrouterina* Fuhrmann, 1912 (Cestoda: Dilepididae).

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Three cestodes representing a species of the genus *Cyclustra* Fuhrmann, 1901, were obtained by the writer from a great blue heron, *Ardea herodias* L., collected at Neville, Rock County, Wisconsin, on April 14, 1947. The bird was an adult male, weighing 2630 grams. Two other great blue herons from this locality were also autopsied, but were negative for this cestode. Two species of *Cyclustra* are known, both from ardeiform birds. The Wisconsin material differs from these in morphological details, and is described herein as new.

The cestodes were stained with Semichon's acetic carmine, and mounted entire. The strobila of this form is thin and nonmuscular, so that morphological details can be easily studied. Serial sections, stained with hematoxylin-eosin, were also prepared.

Cyclustra ardeae n. sp.
(Figs. 1-3)

DIAGNOSIS: Strobila length about 100 mm.; much attenuated anteriorly; greatest width, attained in gravid segments, 2 mm. All segments wider than long; strobilar margins serrate. Scolex about 300 μ in diameter, distinctly set off from well-developed neck; rostellum armed with about 30 hooks ranged in two rows. Large hooks measure about 43 μ, and small hooks measure about 33 μ in length. Ventral longitudinal excretory canals prominent; about 70 μ wide in mature segments. Ventral canals in usual ventral position on poral side, but in dorsal position on aporal side. Genital ducts dorsal to longitudinal excretory canals. Genital pores unilateral, situated in first 1/3 of segment margin; genital atrium prominent and relatively deep. Ovoid to piriform cirrus sac dorsal and somewhat anterior to vagina; it measures 130 to 145 μ long by about

60 μ wide. Internal seminal vesicle apparently absent; external seminal vesicle absent. Vas deferens much coiled near anterior margin of segment, just aporal to end of cirrus sac. Spherical testes measure about 40 μ in diameter, with 40 to 65 per segment. Greater number of testes distributed uniformly in posterior half of segment, behind female genital organs; 8 to 15 testes situated in anterior half of segment, aporal to coiled vas deferens; latter form completely isolated group anterior to female genital organs. Vagina ventral and partly posterior to cirrus sac; its course mediad is undulating; vagina enlarges between ovary and vitelline gland, forming spherical seminal receptacle. Vitelline gland usually reniform, situated near middle of segment directly behind ovary. Lobulated ovary consists of two lobes of equal size; long axis transverse, connected by narrow isthmus. Uterus first seen as transverse, arched tube about 20 μ in diameter, situated near anterior margin of ovary, and ventral to it. At all stages of development poral end of uterus passes over ventral longitudinal excretory canal, but aporal end of uterus is ventral to this canal. Curve of uterus increases with age; posterior and anterior margins develop sacculations. Gravid uterus fills segment almost completely. Eggs, somewhat distorted in available material, about 40 μ in diameter. Hooks of onchosphere measure 10 and 14 μ in length.

HOST: Great blue heron, *Ardea herodias* L.

HABITAT: Small intestine.

TYPE LOCALITY: Neville, in Rock County, Wisconsin.

TYPE: A slide bearing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum, No. 49358.

DISCUSSION: Two species of the genus *Cyclustra* are known. *C. capito* (Rudolphi, 1819) was originally described from *Platalea leucorodia* (L.), and has been recorded by Krabbe (1869, p. 281) from *Ajaja ajaja* (L.), and by Fuhrmann (1909, p. 29) from *Pseudotantalus ibis* L. *C. fuhrmanni* Clerc, 1906, was described from *Botaurus stellaris* L. and, to the writer's knowledge, has not been reported since the original publication

C. ardeae n. sp. differs from *C. capito* in unilateral position of genital pores, size of hooks, smaller number of testes (80 in *C. capito*), and in shape of uterus (forming a complete circle in *C. capito*).

Although the original material of *C. fuhrmanni* consisted of fragmentary specimens, *C. ardeae* n. sp. can be distinguished on the basis of shape of the uterus (a complete ring in *C. fuhrmanni*). According to Clerc (1906, p. 728), the testes are ". . . très nombreux et répartis dans tout le champ dorsal du proglottis" of *C. fuhrmanni*. The two species may also differ in number of and distribution of testes. Since the scolex is unknown for *C. fuhrmanni*, shape and size of rostellar hooks cannot be compared.

STATUS OF THE GENUS *Dendrouterina* Fuhrmann, 1912

During this study, it was noted that the genus *Cyclustra* has important characteristics in common with the genus *Dendrouterina* Fuhrmann, 1912. Two species of the latter, *D. herodiae* Fuhrmann, 1912, and *D. botauri* Rausch, 1948, are known. They are also parasites of ardeiform birds, and have been reported, respectively, in *Garzetta garzetta* (L.) and *Botaurus lentiginosus* (Montagu). A detailed comparison of these genera seems justified, in view of the fact that they are closely related and occur in the same host-group.

After study of Rudolphi's original material, Fuhrmann (1909, p. 31) defined the genus *Cyclustra* as follows:

"Dilepiniden mit einem Rostellum das mit doppeltem Hakenkranz bewaff-

net. Die Genitalöffnungen regelmässig abwechselnd oder einseitig. Hoden zahlreich und dorsal. Uterus anfangs ringförmig mit sekundären Verzweigungen, in reifen Glildedern das ganze Markparenchym erfüllend."

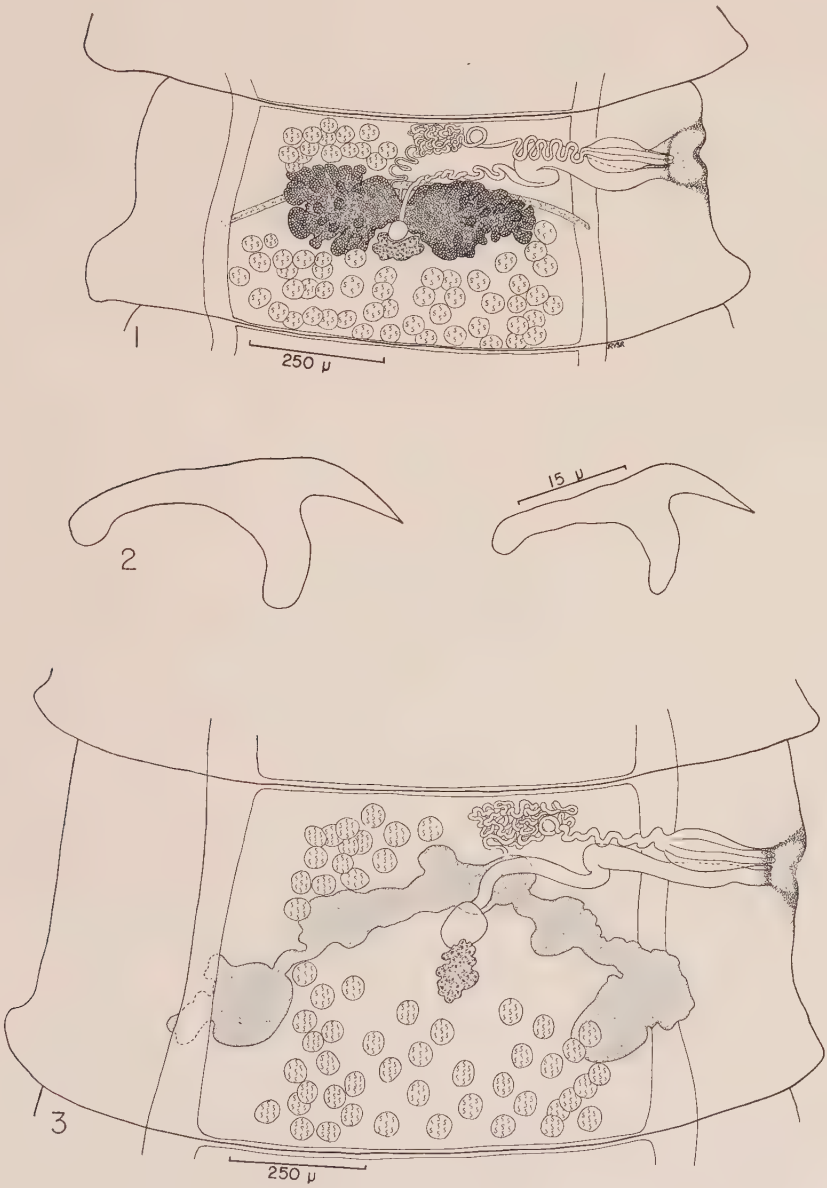

Fig. 1. *Cyclustra ardeae* n. sp. Mature segment; dorsal view.

Fig. 2. *Cyclustra ardeae* n. sp. Rostellar hooks.

Fig. 3. *Cyclustra ardeae* n. sp. Early gravid segment; dorsal view, showing early development of uterus.

Wardle and McLeod (1952, p. 491) incorrectly stated that the genital pores may alternate irregularly in *Cyclustra*.

The scolex of *D. herodiae* is unknown, and the generic diagnosis of *Dendrouterina* was completed by Rausch (1949, p. 76) as follows:

"Scolex well developed; rostellum armed with 2 rows of hooks. Genital ducts dorsal to longitudinal excretory canals. Genital pores unilateral. Testes numerous, situated posterior to female genital organs. Uterus highly branched, with lateral branches passing dorsal to ventral longitudinal excretory canals on poral side, and ventral to it on aporal side. Parasitic in birds."

This diagnosis is inaccurate in one detail. It was stated that the testes are posterior to the female genital organs. This is true in the case of *D. herodiae,* but in *D. botauri* a few testes are situated anterior to the ovary on the aporal side of the segment, as figured by Rausch (1948, p. 432, fig. 1). This point is important in connection with the following discussion.

The species of the two genera have the following characteristics in common: Two rows of rostellar hooks, of similar shape where known (*C. capito, C. ardeae* n. sp., and *D. botauri*); unilateral genital pores (*C. fuhrmanni, C. ardeae* n. sp., *D. botauri,* and *D. herodiae*); highly coiled vas deferens and absence of external seminal vesicle (all 5 species), more or less identical arrangement of female genital organs.

There are actually only two characters which need further consideration; these are distribution of the testes and the shape and relationships of the uterus.

TESTES: The testes are situated dorsally in all five species. In *C. capito* and in *C. ardeae* n. sp., the testes fall into two isolated fields—one anterior and aporal, and one posterior. In both of these most of the testes are distributed across the posterior half of the segment. The testes show a similar distribution in *D. botauri,* but the anterior group of testes is not separated from the larger posterior group. There is no anterior group of testes in *D. herodiae;* rather, the testes are relatively few and all are situated posterior to the ovary and vitelline gland. Information on the distribution of testes in *D. fuhrmanni* is incomplete, so this species cannot be considered here.

It may be seen from the foregoing that the pattern of testicular distribution grades from that seen in *D. herodiae* through *D. botauri* to the extreme seen in *C. capito* and *C. ardeae* n. sp.

UTERUS: The dissimilarities in uterus shape among the five species seem rather great at first glance. However, all are essentially alike. The uterus of *C. ardeae* n. sp. is arc-shaped, and branches develop progressively as the segment becomes older. In *D. herodiae,* the uterus is at first arc-shaped and eventually assumes the shape of an inverted U, designated by Fuhrmann as "hufeisenförmig." The uterus of *D. botauri* is closed at the back, forming a complete circle, but it is otherwise similar to that of *D. herodiae.* In both *C. capito* and *C. fuhrmanni,* the uterus forms a complete ring, and the development of lateral branches is not as advanced.

The placement of the uterus in relation to the ventral longitudinal excretory canals in *C. ardeae* n. sp. seems particularly significant, since it is identical with that of *D. herodiae* and *D. botauri.* No information is available on these relationships in *C. capito* and *C. fuhrmanni.*

It is evident that *C. ardeae* n. sp., *D. herodiae,* and *D. botauri* are very closely related, and differ from one another only in minor details. *C. capito* falls into this group, but may be unique in position of the uterus relative to the ventral longitudinal excretory canals. Presumably *C. fuhrmanni* likewise is morphologically very similar to the four better-known species.

The writer has studied only specimens of *C. ardeae* n. sp. and *D. botauri,* but this, combined with information in the literature on the other three species, has led to the conclusion that these cestodes are congeneric. *Cyclustra* Fuhrmann, 1901, has priority over *Dendrouterina* Fuhrmann, 1912, and must be retained. The diagnosis of the genus *Cyclustra* is emended to include the two additional species.

Cyclustra Fuhrmann, 1901, emend.
(Syn. *Dendrouterina* Fuhrmann, 1912)

DIAGNOSIS: Dilepididae. Rostellum armed with double row of hooks. Genital pores unilateral or regularly alternating. Testes numerous, dorsal; situated mainly posterior to female genital organs, but also anterior to latter in some species. External seminal vesicle absent. Uterus of variable shape, ranging from arc-shaped to ring-shaped, and usually enclosing female genital organs; having numerous branches or extensions when gravid. Uterus dorsal to ventral longitudinal exretory canal porally, and ventral to it aporally. Parasites of ardeiform birds.

Five species are assigned to the genus *Cyclustra* as emended: *C. capito* (Rudolphi, 1819), *C. fuhrmanni* (Clerc, 1906), *C. herodiae* (Fuhrmann, 1912) n. comb., *C. botauri* (Rausch, 1948) n. comb., and *C. ardeae* n. sp.

These cestodes are readily differentiated on the basis of host-species occurrence, form of gravid uterus, and other details, so that a key would at present serve little purpose.

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Discolaimus auritus n. sp. found inhabiting forest soil in Brazil (Nematoda, Dorylaimidae)

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As previously reported by the writer (1953), the genus *Discolaimus* Cobb, 1913 occurs in Brazil, being sometimes obtained in association with other free-living and plant parasitic nematodes. The study of one of the forms found inhabiting wood soil in the State of São Paulo led to the establishment of a new species, here described as *Discolaimus auritus* n. sp.

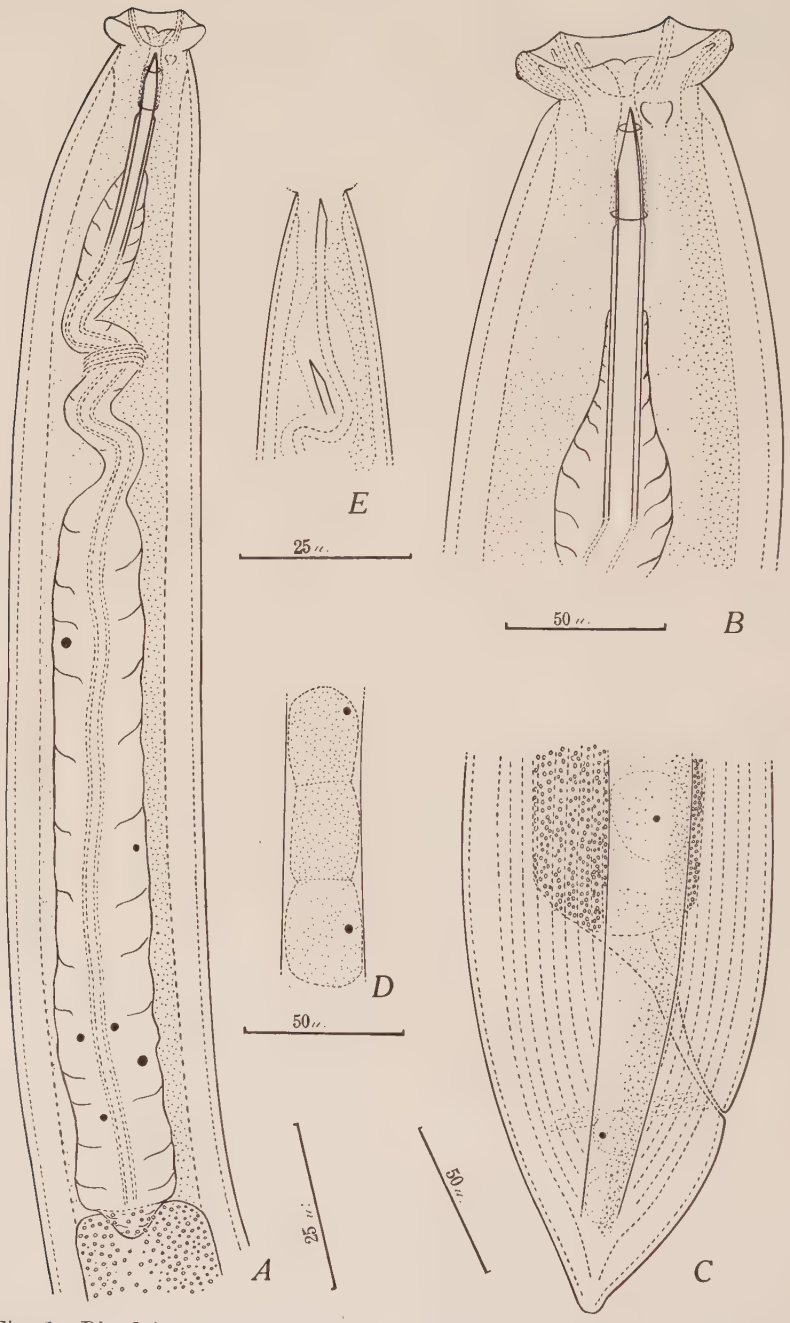

Fig. 1. *Discolaimus auritus* n. sp. A. Oesophagus. B. Head. C. Tail of the female. D. Detail of the lateral field. E. Part of the oesophagus of a larva showing a new spear which would be used in the next moult.

DESCRIPTION. ♀ : 1.64 mm. a = 27.06; b = 4.27; c = 44.1; V = 44.3%.* Body tapering near the extremities. Lip region broadly expanded being half

as wide as body; labial papillae prominent; amphids small, about one-sixth as wide as labial region. Spear 24.9μ long, slightly arcuate; spear extension measuring 41.5μ and spear aperture 11.8μ. Cuticle with fine transverse striations. Lateral fields about one-fourth as wide as mid-body, being formed by a single line of rather large cells (fig. 1 D).

Tail somewhat shorter than anal body diameter, dorsally and ventrally convex-conoid to digitate terminus. No papillae could be seen on the tail. If they exist, they are extremely small.

Oesophagus having an elongate bulb beginning near the middle of the spear extension. The oesophagus soon narrows in order to pass through the nerve ring and then enlarges (fig. 1 A), forming an irregularly cylindrical and muscular basal portion, which measures 236.0 x 34.0μ.

Five nuclei of oesophageal glands could be observed in the basal bulb, as illustrated. Cardia conoid. Cells of intestine with small dark granules. In one larva, the circumference of the intestine comprised at least 6 cells.

Ovaries two, reflexed, equal in development. No eggs were seen in the uterus.

Male unknown. The study of the species has been based on a single female and two larvae, one of which was heavily attacked by an unidentified sporozoan. The tail of the larva is similar to that of the adult, but just a little more acute. The dimension of the two larvae is as follows:

Total length	1.000.0 and 902.0μ
Width	44.0 and 44.0μ
Length of oesophagus	268.0 and 246.0μ
Tail	28.4 and 28.0μ

One of the larvae showed a new spear at the level of the first expansion of the oesophagus, which would replace the former spear during the moult. The new spear was a little longer than the one which would be replaced (20.8 : 20.0μ).

Food habits unknown. It is supposed that it has the same feeding behaviour observed in other species of the genus, which are predacious (Linford, 1937; Linford & Oliveira, 1937).

DIAGNOSIS. *Discolaimus* closely resembling *D. texanus* Cobb, 1913, but differing in: 1. the form of the posterior portion of the body, which is ventrally and dorsally convex and more pointed than in *D. texanus*; 2. the less prominent lips; 3. the greater width of the female (a = 27.06 : 35.0); and 4. the smaller amphids.

HABITAT. The specimens were obtained from soil around the roots of forest trees, in a wood located at Piracicaba, State of S. Paulo, Brazil.

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*a—Length divided by greatest breadth.
b—Length divided by length of oesophagus.
c—Length divided by length of tail.
V—distance of vulva from anterior and expressed as a percentage of total length.

Methylcellulose for the Rapid Preparation of Temporary Nematode Head-Mounts*

E. J. CAIRNS and A. C. TARJAN**

The necessity of critical identification of nematode specimens may require examination of the *en face* view of certain closely related nematodes. The usual method for preparing a head-mount (Buhrer, 1949) is to dehydrate the whole specimen until it is in pure glycerine, then transfer the excised head into molten glycerine-jelly. This method, although yielding a permanent slide, requires the lapse of several weeks before the *en face* preparation of a specimen is ready for examination.

The desirability of a rapid method utilizing a viscous mounting medium into which a specimen could be transferred directly from fixative or water warranted investigation of methylcellulose[1] in this capacity. Occasional use over a period of 3 years has impressed the authors with the speed and ease with which a satisfactory head-mount may be made. Although yielding a temporary mount, this method expedites head-mount preparation thus encouraging its more frequent use as a valuable diagnostic aid.

The viscous, clear medium is prepared by heating 50 ml. of distilled water in a beaker to about 80 to 90°C. Enough solid methylcellulose of one of the higher viscosity types (e.g. 400-4000 cps.) is immersed in the water so that it appears well soaked. After 30 min. the mixture is cooled by partial immersion of the beaker in cold water. If the material fails to dissolve completely the beaker is immersed in ice water. If the material becomes too viscous during or after its preparation, a small quantity of distilled water is judiciously added. The medium becomes slightly more fluid after a few days and bubbles made by stirring disappear.

Methylcellulose medium, prepared in this manner, is well suited for the reception of nematodes or their parts directly from water or fixatives. The specimen is transferred into a drop of the medium on a glass slide, its head is severed, and a coverslip then placed directly on the drop. Manipulation of the coverslip will bring the nematode head into the desired vertical position. The location of the head is indicated in the usual manner by placing three dots, with India ink, in a triangular fashion around the specimen on the glass coverslip. Application of molten paraffin-petroleum jelly mixture to the edges of the coverslip cements it firmly in place so that shifting of the specimen will be greatly reduced. The specimen is now ready for immediate examination under the compound microscope.

This method is specifically applicable as a rapid, routine diagnositic procedure when an *en face* view is desired within a few minutes after the living specimen is examined. The mount is of a temporary nature and is satisfactory mainly for determining the surface structures and the interal sclerotized parts of the nematode's head. These parts, unlike the rest of the nematode's body, apparently are not distorted by this treatment and are the anatomical elements usually considered of diagnostic value in *en face* preparations.

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*Contribution No. 848 of the Rhode Island Agricultural Experiment Station.

**Section of Nematology, Agricultural Research Service, Beltsville, Maryland and Department of Botany-Plant Pathology, Alabama Polytechnic Institute, Auburn, and Department of Plant Pathology-Entomology, University of Rhode Island, Kingston, respectively.

[1]Methylcellulose under the trade name of Methocel in various viscosity types was kindly furnished for experimental purposes by the Dow Chemical Company.

Evaluation of Various Nematodes for Use In Contact Nematocide Tests*

A. C. TARJAN

The submission of diverse chemicals to biological assay necessarily requires the use of suitable test organisms. These organisms normally should be closely related, if not belonging, to species causing the disturbances for which ultimate use of resulting promising chemicals are intended. *In vitro* evaluation of chemicals as contact poisons against various species of nematodes currently is employed as a screening test by several agencies. Those laboratories interested in discovering chemicals for use against plant-parasitic nematodes normally use forms such as *Meloidogyne* sp. or *Ditylenchus* sp. In several cases, mixtures of nematodes, consisting of plant-parasites and non-parasites which normally occur in the soil, are used in lieu of a single species.

In preparation for conducting such bio-assays at the University of Rhode Island, it was reasoned originally that if materials were to be tested as soil nematocides they should be used against soil nematodes of economic importance. Results of numerous examinations of roots and soil from declining perennial plants showed that *Xiphinema americanum* Cobb, 1913, occurred in abundance throughout Rhode Island, as did *Pungentus pungens* Thorne and Swanger, 1936, particularly around roots of forage crops near Kingston, Rhode Island. Several chemical tests had been conducted using these species when it was found that certain members of the "Triton" group of surface active agents[1] were unexpectedly nematocidal at concentrations of 1000 ppm. in aqueous solution. It seemed desirable, in view of these findings to obtain a more accurate knowledge of the behavior of certain nematode species in various aqueous preparations of chemicals. The investigations reported in this paper determined the relative value of several nematodes species for contact nematocide testing.

EFFECT OF SEVERAL TEST NEMATOCIDES ON DIVERSE NEMATODE SPECIES. Since *in vitro* tests had been reported with non-plant parasitic forms such as *Turbatrix aceti* (Müller 1783) Peters 1927 (See Peters 1952) and *Rhabditis* spp. (Smedley, 1936; Uricchio, 1951), a *Diplogaster* sp. obtained from barnyard manure and *Panagrellus redivivus* (Linn. 1767) Goodey 1945, the sour paste nematode, (which also are non-plant parasitic) were tested. The 2 species discussed earlier, *X. americanum* and *P. pungens,* are regarded as suspected plant parasites and were included as such. The remaining nematode species tested, *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949, *Dolichodorus heterocephalus* Cobb 1914, *Rotylenchus erythrinae* (Zimmerman 1904) Goodey 1951, and an undescribed species of *Ditylenchus,* a parasite of mushroom spawn (Cairns, 1953), are all known to be plant parasites.

Earlier tests with Triton surface-active agents had indicated that some of these preparations were nematocidal, therefore 3 members of the group, Triton X-45, Triton X-100 and Triton X-188 were investigated. Also included were Phillips Nematocidal Mixture PN[2] and a dihydroxybenzene. Ten active pre-adult or adult nematodes of each of the species listed (except for *M. incognita* for which second-stage larvae were used) were immersed in aqueous solutions or dispersons of the chemicals at a concentration of 1000 ppm.

*Contribution No. *845* from the Rhode Island Agricultural Experiment Station.
[1]Marketed by the Rohm and Haas Company, Philadelphia, Pa.
[2]Supplied by Larvacide Products, Inc., New York City.

The numbers of nematodes totally inactivated[3] were recorded after immersion at ½-hour intervals for 3 hours and again at 24 hours. Each test was replicated 5 times.

Results of the tests with the 3 Tritons and Phillips Nematocidal Mixture PN were essentially the same, therefore only the graph illustrating the outcome of the trials with Triton X-100 is shown (Fig. 1, A). Perhaps the most striking feature indicated is the extreme susceptibility of *X. americanum* and *P. pungens* to chemical toxicity, since both of these species were totally inactivated in 2 hours or less. *Diplogaster* sp. and *P. redivivus* were only slightly affected while the true plant-parasites were completely unaffected. Figure 1, A clearly illustrates the fallacy in regarding all soil nematodes equal in reaction to a given chemical. When, however, a more effective nematocide such as dihydroxybenzene was utilized, all species succumbed with equal rapidity (Fig. 1, B).

EFFECT OF THREE NEMATOCIDES ON THREE NEMATODE SPECIES. The previous tests clearly indicated that *P. pungens* and *X. americanum* were generally much too susceptible to chemical action for general use as test organisms. Since an adequate supply of test organisms can be insured principally by the establishment and maintenance of cultures, the 3 most easily culturable species of those already investigated, *P. redivivus, M. incognita,* and *Ditylenchus* sp., were selected for further study; these 3 species were about equally resistant to the chemicals tested previously. *Panagrellus redivivus* was cultured on salt-free oatmeal while the root-knot nematode, *M. incognita,* was cultured on living tomato roots in the greenhouse. The mushroom-spawn nematode, *Ditylenchus* sp. was cultivated on mushroom mycelium growing in a compost medium.

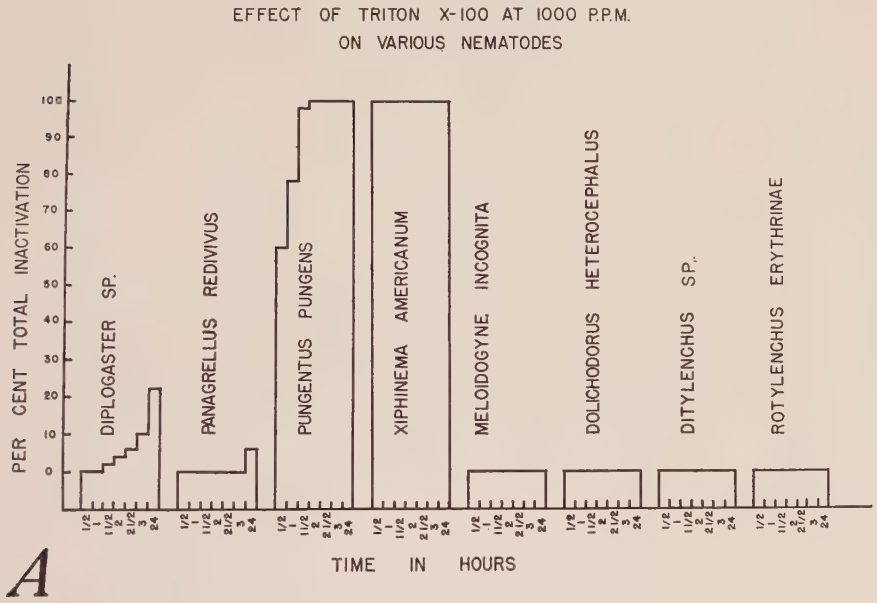
The chemicals utilized were the dihydroxybenzene employed in the previous tests, an unsaturated aliphatic acid found to be nematocidal in earlier trials, and chloropicrin, a well-known and highly effective soil fumigant. Ten active adult or pre-adult nematodes of *Ditylenchus* sp. and *P. redivivus* and 10 second-stage larvae of *M. incognita* were immersed in aqueous emulsions containing 250 ppm. of the chemicals listed above. Each trial was replicated 10 times and the nematodes were observed after ¼, ½, ¾, 1, 1½, 2, 2½, 3 and 24 hours immersion.

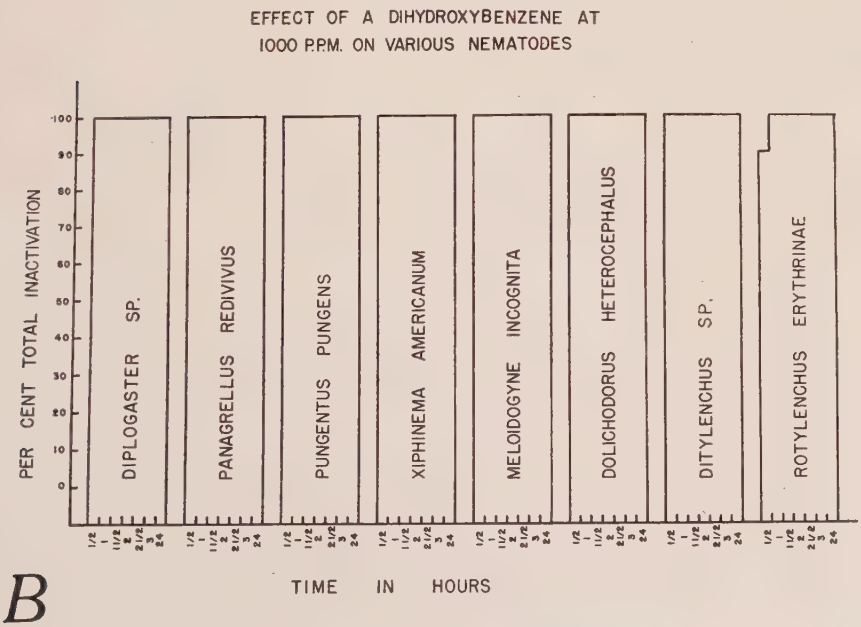
The dihydroxybenzene, as shown in Fig. 2, A, was most toxic to *P. redivivus* in the shortest time while it did not effect all of the *M. incognita* until three hours after immersion. The unsaturated aliphatic acid, on the other hand, was extremely toxic to both *Ditylenchus* sp. and *M. incognita* within ½ hour but never quite inactivated all specimens of *P. redivivus* in all replicates, even after 24 hours immersion. Chloropicrin, strangely, was quite specific in its toxicity for *M. incognita,* but was not particularly lethal to *Ditylenchus* sp. or *P. redivivus.*

Since no one species of the 3 tested, was found to be more susceptible to chemical action than the other two, *Panagrellus redivivus* was tentatively selected as a test organism in preference to the other species. This organism is of a large size (1.04-1.37 mm.) which facilitates rapid manipulation of numerous specimens from the culture medium into test solutions. Cultures of this species can be established simply by inoculating boiled oatmeal with transfer from an old culture. Since the life cycle of this species is very short

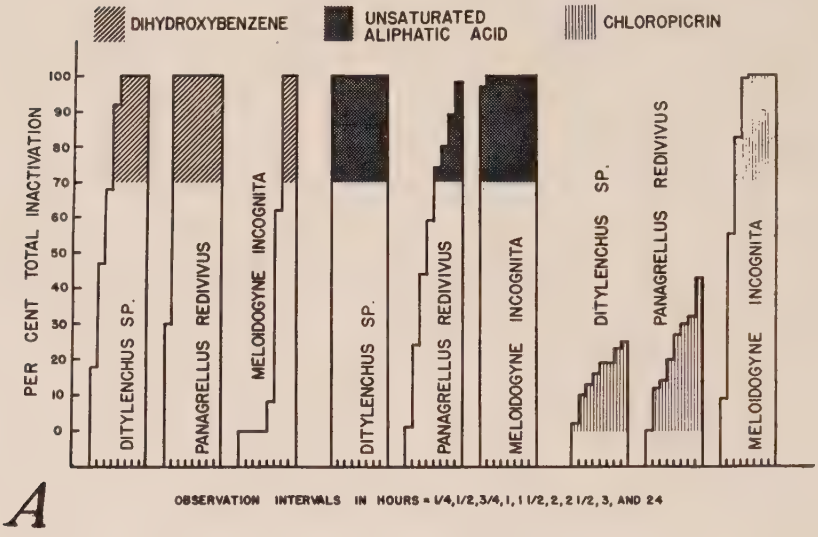
[3]An accurate determination of when death occurs is difficult and would have been subject to error in these tests. Therefore, the designation "total inactivation" was adopted as that condition exhibited by nematodes completely deactivated by a chemical. Nematodes were rated as "totally inactivated" only after they remained immobile following the completion of certain tests designed to induce mobility.

at room temperature, such cultures contain thousands of individuals in all stages of development within a few weeks. *Panagrellus redivivus* is highly active, and this is a considerable advantage since toxic effects of the test chemicals can be evaluated by observing decreased activity.



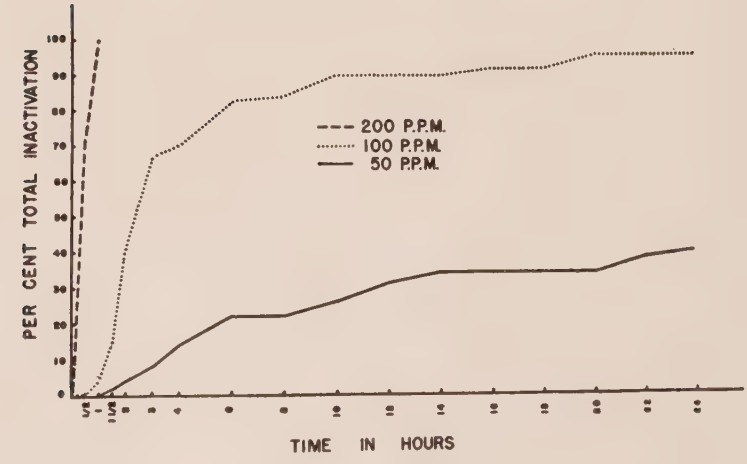


EFFECT OF THREE CHEMICALS AT 250 P.P.M. ON 3 NEMATODE SPECIES



A

TOXIC EFFECT OF THREE CONCENTRATIONS OF A DIHYDROXYBENZENE ON PANAGRELLUS REDIVIVUS



B

EFFECT OF THREE CONCENTRATIONS OF A DIHYDROXYBENZENE ON *P. redivivus*. Having tentatively selected *P. redivivus* as the most suitable organism among those previously tested, it seemed desirable to investigate the reactions of this species to different concentrations of a given chemical over a period of time. The distinct possibility existed that the species may exhibit irregularities in its response to decreasing concentrations of the chemical, thus nullifying its usefulness as a screening test organism.

Aqueous emulsions of the dihydroxybenzene referred to previously were prepared at dosages of 50, 100, and 200 ppm., and 10 specimens of *P. redivivus* were immersed in each concentration. Five replicate trials composed each test. Nematodes were observed at ½, 1, 1½, 2, 3 and 4 hours after immersion and at two-hour intervals thereafter until 24 hours had elapsed from the time the animals were first immersed. The emulsion containing 200 ppm. of the compound totally inactivated all of the nematodes within 1 hour after immersion (Fig. 2, B).

Although the emulsion containing 100 ppm. of the chemical totally inactivated almost 70 per cent of the experimental animals within 3 hours, its affects on the remaining animals were not as pronounced. Even after 24 hours, only an average of 96 per cent of the animals had been totally inactivated. The effects of the 50 ppm. emulsion appear almost as a straight-line curve terminating with only 40 per cent of the animals affected after immersion for 24 hours. A study of the curves shown in Fig. 2, B indicates that reductions in chemical concentration resulted in corresponding reductions in the number of animals totally inactivated within the designated time limits.

SUMMARY

Panagrellus redivivus, Diplogaster sp., *Pungentus pungens, Xiphinema americanum, Meloidogyne incognita, Dolichodorus heterocephalus, Ditylenchus* sp. and *Rotylenchus erythrinae* were compared as to reaction to solutions or emulsions containing 1000 ppm. of either Triton X-188, Phillips Nematocidal Mixture PN, Triton X-100, Triton X-45 or a dihydroxybenzene. *Pungentus pungens* and *X. americanum* were inactivated more readily than the other species tested and accordingly were eliminated from consideration as suitable test organisms. Three of the more easily culturable species, *P. redivivus, M. incognita* and *Ditylenchus* sp. were further investigated in tests using emulsions containing 250 ppm. of the dihydroxybenzene, an unsaturated aliphatic acid, and chloropicrin. No one species was found to be any more susceptible to chemical action than others. The susceptibility of *P. redivivus* to emulsions containing 200, 100 and 50 ppm. of the dihydroxybenzene was found to be proportional to the concentration within the designated time limits.

Panagrellus redivivus is further considered a suitable test organism due to its large size, ease of culture, short life cycle, and active habit of life.

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Dipetalonema procyonis n. sp. from *Procyon lotor lotor* (Linnaeus)

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A single male and two females of a new species of filarial worm were collected from a raccoon on post mortem examination. The raccoon was trapped at the Patuxent Research Refuge at Laurel, Maryland and was one of eight raccoons posted.

DESCRIPTION

Dipetalonematidae Wehr, 1935; Dipetalonematinae Wehr, 1935; *Dipetalonema* Diesing, 1861. Bodies of both sexes taper slightly at the anterior and markedly at the posterior end. Head with eight cephalic papillae in outer circle and two small papillae (internolateral) in the inner circle. Amphids present immediately distal to the latter. The cuticle is finely striated under high magnification. Esophagus consists of an anterior, short muscular and a posterior, long glandular portion. Posterior end of body provided with four lobes. Caudal alae absent.

Male (type) 24.2 to 26.0 mm. long. Lateral width of head at 0.05 mm. from anterior end, 0.11 mm., at nerve ring, 0.12 mm., and at anus 0.058 mm. Esophagus 3.78 mm. long; muscular portion 0.57 mm. and glandular portion 3.21 mm. Deirids and excretory pore located at junction of two portions of esophagus. Nerve ring 0.37 mm. from anterior end of worm. Posterior end of body coiled in four turns. Caudal alae absent. Four pairs of caudal papillae anterior and three pairs posterior to anus. Spicules unequal and dissimilar. Right spicule 0.15 mm. long with a distinct capitulum, calomus, and lamina. Left spicule apparently without capitulum, calomus tubular 0.18 mm. long, lamina thin and whiplike of approximately same length. Gubernaculum absent. Anus 0.145 mm. from posterior end.

Female (paratypes) 34.8 to 37.8 mm. long. Lateral width of head 0.14 mm., measured at 0.05 mm. from anterior end and 0.20 mm. at nerve ring. Dorsoventral width at vaginal opening 0.24 mm. and at anal opening 0.095 mm. Total length of esophagus 2.9 mm., muscular portion 0.52 mm. and glandular portion 2.38 mm. long. Vulva 1.10 mm. from anterior end. Deirids and excretory pore located at junction of two portions of esophagus as in male. Nerve ring 0.28 mm. from anterior end. Posterior end of body turns dorsally at position of the anal opening. Tail 0.4 mm. long with four lobes at tip.

HOST: *Procyon lotor lotor* (Linnaeus).

LOCATION: Subcutaneous connective tissue.

TYPE LOCALITY: Patuxent Research Refuge, Laurel, Maryland.

TYPE SPECIMENS: (1 male and 2 females). U. S. National Museum Helminthological collection. No. 37441

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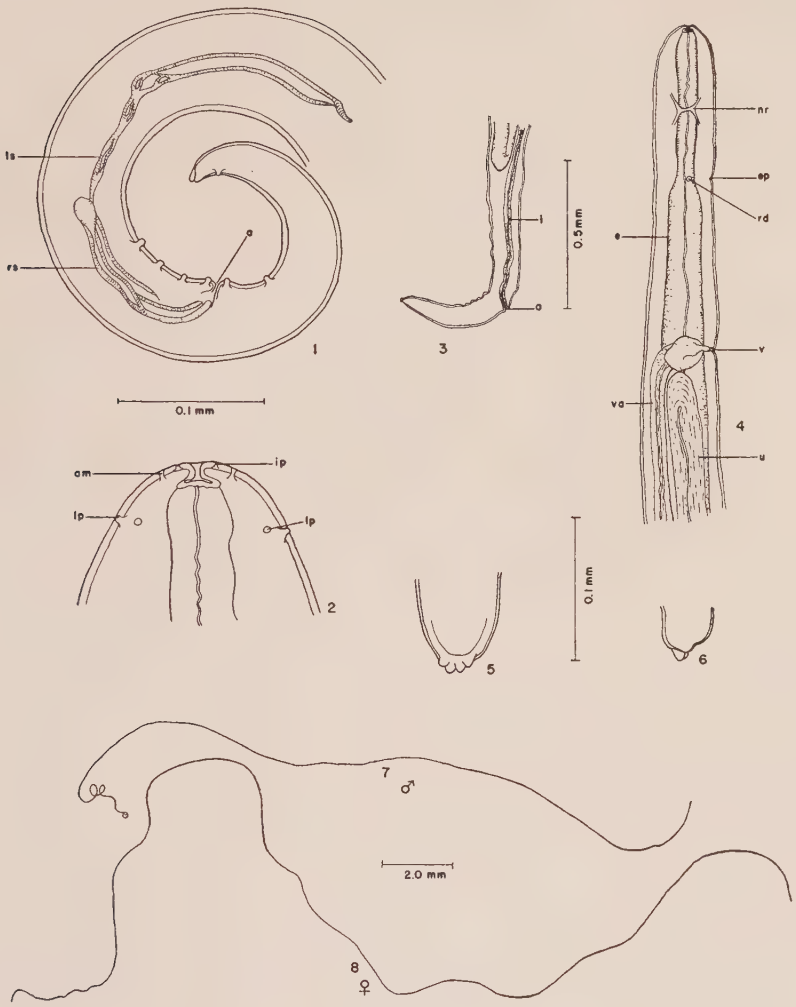
*I wish to express my appreciation to the staff of Patuxent Research Refuge, Laurel, Maryland and particularly to Dr. Carlton M. Herman, Mr. William Good, and Mrs. Miriam Raines of the Henshaw laboratory for providing laboratory facilities and assistance throughout the project, to Mr. Leonard Llewellyn for his assistance and for supplying most of the animals examined, and to Mr. Oscar Warbach for supplying some of the animals examined.

In addition I wish to express my appreciation to Dr. Everett E. Wehr and Mrs. M. B. Chitwood of the Animal Disease and Parasite Research Branch, Agriculture Research Service, U. S. Department of Agriculture, Beltsville, Maryland for their help in the identification and study of this nematode.



Figures were drawn with the aid of a microprojector.

Abbreviations used:

a, anus; am, amphid; e, esophagus; ep, excretory pore; i, intestine; ip, interlateral papilla; lp, lateral papilla; ls, left spicule; nr, nerve ring; rd, right deirid; rs, right spicule; u, uterus; v, vulva; va, vagina.

Figure 1. Right lateral view of posterior end of male.
Figure 2. Dorsal view anterior extremity of male.
Figure 3. Right lateral view posterior end of female.
Figure 4. Right lateral view anterior end of female.
Figure 5. Ventral view posterior extremity female.
Figure 6. Lateral view posterior extremity female.
Figure 7-8 Line tracings of male and female showing comparative lengths.

TABLE I.—A Comparison of Measurements of the Adults of *Dipetalonema Procyonis* n. sp. and *Dipetalonema dasyuri* (Johnston & Mawson 1938)

	D. procyonis	*D. dasyuri*
Males		
Total length	24.2-26.0	20.0-40.0
Length esophagus	3.78	
anterior portion	0.57	0.29-0.32
posterior portion	3.21	1.1-1.2
Length of tail	0.14	0.4-0.5
Nerve ring from head	0.37	0.16-0.22
Right spicule	0.15	0.12
Left spicule	0.36	0.20
Females		
Total length	34.8-37.8	83.0-99.0
Length esophagus	2.9	
anterior portion	0.52	0.28-0.38
posterior portion	2.38	1.7-2.1
Vulva from head	1.10	*
Nerve ring from head	0.28	0.21
Length tail	0.39	0.35-0.70

*Given as a ratio to body length; 1:15 (in young females) and 1:12. A comparative figure in *D. procyonis* is about 1:32.

Discussion

A review of the genus *Dipetalonema* Diesing, 1861, as amended by Chabaud (1952), indicated that the worms from the raccoon had not previously been described. Morphologically the new species is most like *D. dasyuri* Johnston and Mawson, 1938, from *Dasyurus viverrinus*. For comparison, the measurements of the adults of the two species are presented in Table I. The most marked differences are seen in total length and position of the vulva in the females, length of the left spicule in the males, and the length of the posterior portion of the esophagus in both sexes. In addition to these differences, *D. dasyuri* has only two dorsal cephalic papillae and two caudal lobes. The male of *D. dasyuri* has only four pairs of caudal papillae, two preanal and two postanal.

Summary

Dipetalonema procyonis n. sp. is described from specimens obtained from the subcutaneous connective tissue of a raccoon. The species is similar to *D. dasyuri* but differs in number and arrangement of cephalic papillae and number of lobes on the tail; position of the vulva of the female; and the length of the spicules, and numbers and arrangements of the caudal papillae in the male.

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Some Cestode Parasites of the Old-Squaw, *Clangula hyemalis* (L.)

EVERETT L. SCHILLER*

Since 1949, when a survey of helminth parasites of vertebrates was undertaken in Alaska by the Animal-borne Disease Section of this Center, a total of 116 old-squaw ducks, *Clangula hyemalis* (L.), has been collected and examined. These ducks, comprising 84 females and 32 males, were taken at all seasons of the year and included birds of all ages, with the exception of non-flying young. Seventy-one old-squaws were collected by the writer during January of 1954, on St. Lawrence Island, where enormous numbers of these winter sea ducks constitute an important source of food for the Eskimo during the time of the year when marine mammals, upon which they normally depend for sustenance, are usually unavailable or difficult to obtain. Other localities in Alaska from which old-squaws were collected include Pt. Barrow, Anaktuvuk Pass, Kotzebue, and Arctic Village.

The autopsies disclosed that, of the total number, 68 (59%) of these ducks were parasitized by cestodes. The species of cestodes found, in order of the frequency of their occurrence, are as follows: *Lateriporus teres* (Krabbe, 1869); *Hymenolepis jägerskiöldi* Fuhrmann, 1913; *Fimbriaria fasciolaris* (Pallas, 1871); *Hymenolepis coronula* (Dujardin, 1845); and *Fimbriarioides intermedia* (Fuhrmann, 1913). All of these species have been recorded previously from North America; however, the occurrence of *L. teres, H. jägerskiöldi,* and *F. intermedia* in *Clangula hyemalis* constitutes new host records.

Only 4 immature birds were collected on the nesting grounds. These were taken with the adult female at the mouth of the Noatak River near Kotzebue by Dr. Robert Rausch of this laboratory. The adult bird harbored both *L. teres* and *F. fasciolaris,* while the young contained only *L. teres.*

Some notes on the taxonomic status of *L. teres* and *F. intermedia,* together with a description of the scolex of the latter species, were presented in a previous paper by the writer (1954). The other species of cestodes obtained in this study have been, for taxonomic purposes, adequately described in the literature and it is therefore considered unnecessary to discuss them further here.

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Trichostrongylosis in Cattle

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The purpose of the present paper is to report the occurrence of *Trichostrongylus colubriformis* and *T. axei,* together with that of some other nematodes, in a herd of cattle in Northern Virginia and the apparent effects of this nematode infestation.

REVIEW OF LITERATURE

A considerable number of species of the roundworm genus *Trichostrongylus* are known to parasitize many different animals and some of these species are injurious parasites of domestic ruminants, particularly goats and sheep. The species of this genus most commonly found in domestic ruminants in North America are *T. axei, T. colubriformis, T. vitrinus,* and *T. capricola. T. axei* and *T. colubriformis,* the former occurring principally in the abomasum or fourth stomach, and the latter in the small intestine, have been collected from all domestic, and some semi-domesticated and wild ruminants, in North America. *T. vitrinus* occurs principally in sheep and goats, and there are no records of its having been collected from cattle in North America. Roberts (1939 a and b), however, reported its occurence in cattle in Australia, and the finding of 16 specimens in one animal. Apparently, therefore, it is not a common species in cattle. *T. capricola* has been reported from both sheep and goats, but, as its name indicates, it is more common in goats than in sheep.

T. axei is the species most commonly encountered in cattle and is considered by most authorities to be the only one of any importance as a pathogen. Roberts (*loc. cit.*) reported that infestations usually did not exceed 15,000 worms, but found 76,000 worms in one case, and he cites Taylor's (1934) finding of 140,000 worms in a two-year-old heifer suffering from parasitic gastritis. Andrews *et al.* (1953) reported *T. axei* as a common parasite of cattle in the Southeastern United States; the maximum number of *T. axei* recovered from one animal was 400,000 from a mature Brahman cow. Reports on the occurrence of *T. colubriformis* in cattle are relatively few. Most of them merely mention finding this worm in cattle, and they furnish no information on the effect of infestation on cattle. Walker (1925) reported finding it in young cattle in Kenya and stated that his report was apparently the first record of the occurrence of this roundworm in cattle. MacDonald (1932) mentioned its occurrence in cattle in Northern Rhodesia and noted that it was not common. Sprent (1946) reported it from Zebu cattle in Northern Nigeria. Krupski and Uehlenger (1942) stated that they found specimens of *T. colubriformis* in the fourth stomach (?) of a calf in Switzerland. Roveda and Riguelet (1947) reported finding it in cattle in Argentina, and there are some additional records of its occurrence in cattle from Russian sources. There are, so far as we are able to determine, only three published records of the occurrence of *T. colubriformis* in cattle in North America. Woodhouse, Reid and Schmidt (1940) reported it from

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*Retired May 31, 1953.

**The writers express appreciation to Dr. C. A. Manthei of this Branch, who referred the original material to us and aided in the field observations of the affected cattle.

cattle in the Gulf Coast area of Texas. Porter (1942) found this parasite in seven of 81 cattle examined; six of the animals came from the vicinity of Auburn, Alabama, and the seventh from Athens, Georgia. Five of the animals positive for this parasite ranged in age from one to six months and two were yearlings. The calves harbored from 100 to 750 worms of this species, while one of the yearlings harbored 200 and the other 9,700. Baker (1949) also reported recovering *T. colubriformis* from calves in New York State.

LABORATORY AND FIELD OBSERVATION

In the late summer of 1950, 38 six- to nine-month-old Santa Gertrudis calves were brought from Texas onto a farm in Northern Virginia. They were kept in small lots around the barn and rack fed during the winter of 1950-51, and in June of 1951 were placed on pasture. Shortly thereafter many of the animals, now from 15 to 18 months of age, began to scour and lose weight. Five of them died or were killed *in extremis* before August 4, 1951. Two of these animals were brought to the Animal Disease Laboratory, Beltsville, Md., in a moribund state for examination and diagnosis. One of these died the day after its arrival at the laboratory, and the other was killed *in extremis*. Johne's disease was suspected, but a thorough examination failed to reveal any evidence of this disease. When the examination of the second animal failed to reveal any bacterial or viral cause of the condition from which these animals had been suffering, one of the examiners thought of the possibility that worm parasites might be involved. Unfortunately, the parts of the carcass needed by us to determine whether or not worm parasites might be responsible for the condition of the animal had been discarded except for a small quantity (about 50 cc.) of the mixed fluid contents of the small intestine and cecum. This material was examined and less than 50 worms were recovered. These were identified as *T. colubriformis, Cooperia punctata, C. oncophora,* and *C. pectinata,* the predominant species present being the first mentioned. This fluid material was watery with little or no solid material. Microscopic examination showed that this fluid contained about 430 worm eggs per cc., 406 of these being eggs of *Trichostrongylus* spp. and 24 those of *Cooperia* spp.

The owner of the herd was notified of the results of the examination and the farm was visited for the purpose of examining the remaining animals. On arrival at the farm it was learned that two more animals had died, making the total number of deaths seven up to August 7. The herd had been divided into two groups, one group containing those animals that were recovering or least affected and the other the animals still showing clinical symptoms. The animals in this second group were thin and some of them were still scouring profusely. Attempts were made to collect some fecal samples from both the severely and the apparently less severely affected animals in this group, but it was difficult to approach them on foot. The samples that were obtained had to be picked up from the soil as they were discharged. This offered some difficulties in the cases where the discharges were watery. Fecal samples were also obtained from a few animals in the group that had recovered and no longer showed clinical symptoms except for being of poor weight considering their age and breed. The results of examination of these fecal samples for evidence of parasitism are presented in table 1.

TABLE 1.—Results of Examination of Fecal Samples

Animal Number	Condition of Feces	Parasite Eggs Per cc* Trichostrongylus	Cooperia	Oesophagostomum	Capillaria
Group I.—Condition poor—some animals scouring					
?	Fluid	78	6	–	–
30	Soft	347	2	4	1
42	Watery	630	275	–	–
53	Soft	100	10	–	–
Group II.—Condition fair—no scouring					
33	Normal	3	–	1	–
36	do	3	2	–	–
48	do	8	4	–	–

*A few coccidia oocysts were seen in these fecal samples.

These results indicate that members of the genus *Trichostrongylus* were the predominant worm parasites present in the affected animals at the time the examinations were made. However, since it is difficult to determine the species of *Trichstrongylus* present on the basis of the eggs found in the feces, and no abomasal contents were available from the autopsied animals so that adult worms could be identified, it was decided to culture a composite sample of the feces collected and to give the resulting larvae to a parasite-free lamb, no parasite-free calves being available. The culture yielded 5,330 infective larvae of *Trichostrongylus* spp. and *Cooperia* spp. in about the same proportional numbers as the egg counts. These larvae were administered to the lamb in two doses, one on September 24 and the other on October 10. A maximum fecal egg count of 82 per gram was reached on November 12. The lamb was killed on November 27 and the following worms were recovered: *Ostertagia ostertagia*—2, one male and one female (this species is normally found in cattle); *Trichostrongylus axei*—139, 86 males and 53 females; *T. colubriformis*—28, 19 males and 9 females.

Discussion

It was, of course, unfortunate that an accurate count of the number of worms in the autopsied animals could not be made because the entire gastrointestinal tract of these animals was not made available for examination for parasites. However, Roberts (1942) reported finding 702 specimens of *Trichostrongylus* (609 *T. colubriformis* and 12 *T. axei*) in a calf that had a fecal egg count of only 12 per gram. This count was made on presumably normal cattle feces, and, assuming it to be representative, it would indicate that at least two of the egg counts recorded above might have resulted from infestations of 20,000 to 35,000 or more trichostrongyles, if the feces had been of normal consistency. However, the feces of the two animals showing the highest egg counts was fluid, and, therefore, it is not unreasonable to assume that the number of worms present in these animals was far in excess of the numbers mentioned. The number of *Trichostrongylus* spp. eggs found in the feces of the experimentally infected lamb harboring 167 adult parasites at autopsy cannot be taken as a measure for estimating the number of worms harbored by cattle for obvious reasons.

Since two species of Trichostrongylus, namely *T. axei* and *T. colubriformis,* were present in these cattle as shown by their recovery at necropsy

from the experimentally infected lamb, it is impossible to say which one of these was chiefly responsible for the condition of the affected cattle. Parasitic gastritis and diarrhea caused by *T. axei* have been described by Stewart and Croften (1941), Gorrie (1943), Plato Guerrero (1931), and Andrews *et al.* (1953). According to Stewart and Croften the disease develops in the winter when the animals are kept on a bare minimal maintenance ration although the infestation is acquired on pasture during the previous grazing season. It affects cattle between the ages of one to two years and the symptoms are diarrhea, wasting, loss of appetite, etc. Gorrie also states that it occurs in young cattle and the title of his paper indicates the symptoms shown by infested animals. As previously stated, published reports of the occurrence of *T. colubriformis* in cattle are few and there are apparently no reports of the effect on cattle of infestation with this parasite, but it can be tentatively assumed, until more information is forthcoming, that this species in cattle, under favorable circumstances, may cause clinical symptoms similar to those it produces in sheep and goats.

CONCLUSION

The available evidence indicates strongly that the death losses in this herd, and the clinical symptoms shown by many of the animals, were due to worm infestation, and that the worm parasites principally responsible were those of the genus *Trichostrongylus,* with both the abomasal and intestinal species present, and with perhaps minor assists from lesser numbers of ostertagias and cooperias.

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Helminths of Salamanders from Promised Land State Forest Park, Pennsylvania*

JACOB H. FISCHTHAL

During late July and early August, 1954, 111 salamanders were collected from three localities within Promised Land State Forest Park, located in Pike County, in the Pocono Mountains of northeastern Pennsylvania. This natural forest area of 2,328 acres is 1,700 feet above sea level, surrounding the 420 acre Promised Land Lake lying within the Delaware River watershed. All salamanders were immediately preserved in 3 percent formalin and were examined within the following three weeks.

Of the 111 salamanders taken, 38 or 34.2 percent were infected with at least one species of helminth. Hosts examined were 14 efts or terrestrial stage of the newt, *Triturus v. viridescens* (11 or 78.5 percent infected), one terrestrial spotted salamander, *Ambystoma maculatum* (uninfected), 24 terrestrial red-backed salamanders, *Plethodon c. cinereus* (5 or 20.8 percent infected), 1 aquatic larval purple salamander, *Gyrinophilus p. porphyriticus* (uninfected), 14 terrestro-aquatic two-lined salamanders, *Eurycea b. bislineata* (2 or 14.2 percent infected), and 57 terrestro-aquatic dusky salamanders, *Desmognathus f. fuscus* (20 or 35.0 percent infected). From the above hosts 7 different species of worm parasites were recovered, 3 of which were trematodes, and 4 nematodes. No cestodes were found.

HELMINTHS RECOVERED

The figures in parentheses, following the name of the host under each species of parasite listed immediately below and after each parasite in the discussion, represent the following: first figure—total number of individuals harboring that parasite; middle figure—percent infection; last figure—average number of worms per host. All helminths reported in this study probably represent new distribution records.

A. TREMATODA

1. *Brachycoelium salamandrae* (Froelich, 1789) Dujardin, 1845.

HOSTS: *Plethodon c. cinereus* (5-20.8-3.2), *Eurycea b. bislineata* (2-14.2-10), *Desmognathus f. fuscus* (11-19.3-2.5).

POSITION: Small intestine.

Of the 5 infected *P. c. cinereus,* 2 had 1 adult worm each, while the other 3 had 2, 4 and 8, respectively. The 2 infected *E. b. bislineata* harbored 8 and 12 adult worms, respectively. Four of the 11 *D. f. fuscus* were infected with 1 adult worm each, 3 with 2 each, 3 with 3 each, and 1 with 8. This parasite

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has a world-wide distribution and has been reported many times from amphibians and reptiles. Its life cycle probably is associated with a terrestrial habitat.

2. *Phyllodistomum solidum* Rankin, 1937.
   HOST: *Desmognathus f. fuscus* (10-17.5-1.9).
   POSITION: Urinary bladder.
   Six *D. f. fuscus* had 1 adult worm each, while 1 had 1 immature specimen; 1 was infected with 3 immature worms, 1 with 2 adult and 2 immature, and 1 with 1 adult and 4 immature. This parasite is known to have a strict host-specificity, although Groves (1945) reported its accidental occurrence in 1 of 97 *Eurycea b. bislineata*. *P. solidum* requires an aquatic habitat for its life cycle.
3. *Plagitura salamandra* Holl, 1928.
   HOST: *Triturus v. viridescens* (8-57.1-7.1).
   POSITION: Small intestine.
   One *T. v. viridescens* was infected with 2 adult worms. The remaining 7 harbored immature specimens as follows: 2 with 4 each, 2 with 5 each, and 1 each with 6, 9 and 22, respectively. This parasite exhibits a fairly strict-host specificity, although its rare occurrence in two other species of salamanders has been recorded by Rankin (1937). *P. salamandra* requires an aquatic habitat for its life cycle.

B. NEMATODA

1. *Capillaria tenua* Mueller, 1932.
   HOST: *Triturus v. viridescens* (3-21.4-1).
   POSITION: Mucosa of small intestine.
   Two of the infected newts harbored 1 adult worm each, while the third possessed an immature specimen. According to Rankin (1937), this parasite is also occasionally found in frogs. *C. tenua* appears dependent upon an aquatic habitat for completion of its life cycle.
2. *Cosmocercoides dukae* (Holl, 1928) Wilkie, 1930.
   HOSTS: *Triturus v. viridescens* (1-7.1-1), *Desmognathus f. fuscus* (2-3.5-1.5).
   POSITIONS: Small and large intestines.
   T. v. viridescens harbored a single specimen in the lower small intestine as did 1 *D. f. fuscus*. The other dusky salamander had 2 worms in its large intestine. *C. dukae* has been recorded from many terrestrial and aquatic amphibians and reptiles in the United States and Canada.
3. *Oswaldocruzia pipiens* Walton, 1929.
   HOST: *Desmognathus f. fuscus* (1-1.8-1).
   POSITION: Stomach.
   This parasite has been listed from many species of amphibians and reptiles in the United States. It apparently is more common in hosts associated with an aquatic habitat.
4. Spirurid cyst
   HOST: *Triturus v. viridescens* (1-7.1-1).
   POSITION: Esophagus.
   A single cyst was found embedded in the wall of the esophagus.

DISCUSSION

Kelley (1934), in a study of trematode parasites of 144 *Triturus v. viridescens* from Seaton's Lake and Gorley's Lake near Uniontown in southwestern Pennsylvania, recorded *Brachycoelium salamandrae,* larval *Diplostomulum trituri, Gorgoderina intermedia,* and *Plagitura salamandra.* In addition Kelley mentioned obtaining from Seaton's Lake a larval tapeworm

(probably *Ophiotaenia* plerocercoids), and adult *Bothriocephalus rarus.* A nematode, *Capillaria* sp., was taken by her from both lakes. Kelley commented on the great variation in size and various morphological structures shown by *Plagitura salamandra* and ". . . ventures to suggest that *Plagitura parva* might be included within this group." Stunkard (1936) contended that by recognition of two species of *Plagitura,* namely, *salamandra* and *parva,* the variations reported by Kelley could be explained. Therefore, it is probable that *P. parva* is also found in newts of southwestern Pennsylvania. Stunkard reported *P. parva* in 24 of 27 newts from a pond in the Pocono Mountains, Pennsylvania. The present author did not find *P. parva* in the efts examined from Promised Land State Forest Park in these same Pocono Mountains. Stunkard further mentioned taking *P. salamandra* from newts in Pennsylvania, but the locality is not cited.

Fischthal (1955) examined salamanders for worm parasites from Delaware County, New York, approximately 55 miles north of Promised Land State Forest Park and also within the Delaware River watershed. The report does not separate data relating to the Susquehanna River and Finger Lakes watersheds from the above because the findings, where sufficient hosts were examined, were fairly comparable for all three. However, it now may be recorded that from within the Delaware River watershed of New York, the following parasites were recovered: From 46 aquatic *Triturus v. viridescens* (45 or 97.8 percent infected) were taken *Gorgoderina attenuata* (9-19.6-2.7), *Megalodiscus temperatus* (18-39.1-3.6), *Plagitura salamandra* and *parva* (26-56.5-8.4), Metacercaria (4-8.7-17), *Bothriocephalus rarus* (7-15.2-4.6), *Capillaria tenua* (18-39.1-3), *Cosmocercoides dukae* (2-4.3-1), and Spirurid cyst (8-17.4-1.6); from 3 terrestro-aquatic (adult) *Gyrinophilus p. porphyriticus* (2 or 66.7 percent infected), *Spironoura* sp. (2-66.7-6); 6 *Plethodon c. cinereus,* 5 *Eurycea b. bislineata,* and 7 *Desmognathus f. fuscus* were negative.

Multiple infections in salamanders from Promised Land State Forest Park were scarce. In *T. v. viridescens* one harbored 4 immature *Plagitura salamandra* and 1 *Capillaria tenua,* while a second possessed 5 immature *P. salamandra* and 1 encysted larval spirurid. In *D. f. fuscus* one was infected with 1 *Brachycoelium salamandrae* and 1 *Phyllodistomum solidum,* a second with 8 *B. salamandrae* and 1 *Cosmocercoides dukae,* a third with 1 *P. solidum* and 2 *C. dukae,* and a fourth with 3 immature *P. solidum* and 1 *Oswaldocruzia pipiens.* Multiple infections with as many as 4 different helminths were common in *T. v. viridescens* from Delaware County, New York, occurring in 28 of the 46 examined. However, these were all aquatic stages of the newt, whereas those of the present study were all efts or the terrestrial stage. Perhaps after these efts returned to an aquatic habitat, they too would exhibit more instances of multiple infections.

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A Method of Culturing Plant Parasitic Nematodes Under Sterile Conditions*

W. B. MOUNTAIN**

The relationship of parasitic nematodes to root rot diseases of plants must often be judged largely upon conjecture due to the difficulty of proving by recognized pathological methods whether or not the disease results from the nematode alone or develops from the interaction of nematodes and other organisms. In order to apply the rules of proof of pathogenicity to any organism, one must first obtain the suspected pathogen in pure culture, preferably the progeny of a single isolate, induce the disease with this culture and extract the same organism after the normal symptomatological picture appears. The first requirement in applying this desirable procedure to nematode diseases involves culturing the nematodes so as to obtain a pure line separate from fungi and bacteria. The method should be such that the nematodes could be observed frequently, that the cultures could be kept free of bacterial and fungous contaminants and that material could be removed readily for study or host inoculation. In other words, the ideal would be the growing of nematodes in sterile media under sterile conditions in laboratory glassware.

This paper reports the successful culturing of the plant parasitic nematode *Pratylenchus minyus* Sher and Allen, in petri dishes for periods of over four months, under aseptic conditions, using root tissue cultures. An undescribed species of *Pratylenchus,* obtained from roots of red clover was also successfully cultured, but for a shorter period of time.

References to attempts at culturing plant parasitic nematodes are not numerous and in no case have they been cultured on a non-living medium. A number of soil-dwelling nematodes, particularly of the genus *Rhabditis,* have been cultured under axenic conditions. Thus Dougherty and Calhoun (1948) sterilized these nematodes by use of penicillin, streptomycin and merthiolate, and then reared them on sterile agar media containing liver extract. In the case of plant parasitic nematodes, there are several reports of culturing these organisms under sterile conditions on roots of seedlings. Byars (1914) sterilized eggs of a root knot nematode (*Meloidogyne* sp.) and successfully inoculated roots of tomato and cowpea seedlings which were growing in Preffer's nutrient agar in test tubes. Tyler (1933) also cultured root knot nematodes aseptically on seedlings of tomato, sterilizing the nematodes by hydrogen peroxide. Hastings and Bosher (1938) obtained infection of oat roots by the meadow nematode *Pratylenchus pratensis* (deMan, 1880) Filipjev, 1936 under sterile conditions. They placed segments of oat roots infested with this nematode in a petri dish filled with peat moss soaked with a nutrient solution. The nutrient medium contained 0.1% malachite green, which is an effective fungicide and bactericide at that concentration. Oat seeds were placed in this medium and upon their germination, the nematodes moved from the root segments, through this fungicidal medium, into the roots

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of the oat seedlings. These nematode-infested roots proved to be free from the fungi and bacteria naturally associated with the nematode in field infection. The technique of using the roots of seedlings as a substratum for culturing nematodes, however, has several inherent disadvantages. The first is that photosynthesis must be maintained if the roots are to remain alive and therefore light must be provided. Allowance must be made for the growth of the aerial portions of the seedlings, hence much larger containers must be used and it is difficult to maintain an entire plant under sterile conditions for any considerable length of time.

DESCRIPTION OF THE NEW METHOD

In the method described herein, only the roots are used and thus the culture can be maintained indefinitely in standard petri dishes. Also, no light is needed and the cultures can be stored in an incubator at a constant temperature. Initially, the method was designed for the building-up of pure-line nematode inoculum, but certain modifications have extended its possibilities until it may now be used also for studies of penetration and subsequent colonization of the nematodes in the absence of any other microorganism, of life cycles and host ranges, relationships between nematodes and other microorganisms, and, with further modification, of physiological responses to nematode invasion. The technique may accordingly be regarded as an important one in nematode disease investigations.

White's culture medium is used without modification (White 1943), except that the solution is made up into a 0.75% agar medium, using well-washed shredded agar. This agar medium is autoclaved and poured into sterile petri dishes in the normal fashion. The formula for White's nutrient medium is as follows:

Salts	Mg./liter
$MgSO_4$	360.0
$Ca(NO_3)_2$	200.0
Na_2SO_4	200.0
KNO_3	80.0
KCl	65.0
$NaH_2PO_4.H_2O$	16.5
$Fe_2(SO_4)_3$	2.5
$MnSO_4$	4.5
$ZnSO_4$	1.5
H_3BO_3	1.5
Nicotinic acid	0.1
Sucrose	20,000.0
Glycine	3.0
Nicotinic acid	0.5
Pyridoxine	0.1
Thiamin	0.1

Thus far the roots of the three plants, corn, tobacco and red clover, have been used in culture. Seeds were surface-sterilized in commercial sodium hypochlorite solution, diluted to give an average chlorine concentration of 1%, for a period of 15 minutes for corn and 10 minutes for tobacco and red clover. These seeds were then placed on moist sterile filter paper in sterile petri dishes. After germination, the root tips were severed by sterile scissors,

transferred to the nutrient agar in petri dishes, and were then ready to receive the nematode inoculum.

Corn growing in nematode-infested soil in the greenhouse served as the source of inoculum for *P. minyus*. The corn roots, after washing, were placed in the Baermann funnel and after six to eight hours about 40 cc of water were removed, decanted and the residue placed in a Syracuse watch glass. If the nematodes were left in the bottom of the funnel for longer periods of time, many of the parasitic forms were killed or became inactive. With the aid of a dissecting microscope, using a magnification of 30X, approximately 30 mature females were removed singly by a bamboo pick and placed in a drop of sterile water in a hollow-ground microscope slide contained in a petri dish.

All subsequent transfers were carried out under sterile conditions in a transfer room under a source of ultraviolet light, to which all equipment, including the dissecting microscope, had been exposed for one hour prior to use. All of the baths were hollow-ground microscope slides within petri dishes which had previously been sterilized in an oven at 350°F for three hours.

The nematodes were removed singly from the first water bath to a second, sterile-water bath, using a special sterile bamboo pick. Next, the nematodes were transferred singly, by means of the pick, to a 0.1% solution of streptomycine sulphate for at least 15 minutes. Neither the time of exposure nor the concentration used appeared to be too critical as the nematodes were not affected by this substance, even at ten times this concentration.

Following this treatment, the nematodes were placed through one further sterile water bath, from which they were removed in bulk by means of a very fine sterile pipette and placed in the petri dish beside the root culture. All cultures were incubated at 30°C, in darkness. By inverting the petri dish, the cultures could be studied in detail under the compound microscope.

Application of Method

This technique has been used in studying the relationship of nematodes to brown root rot of tobacco on Ontario. The complete details of this study are published elsewhere (Mountain, in press). It was possible to induce lesions and killing of cultured tobacco roots by the feeding of *Pratylenchus minyus,* in the absence of soil fungi or bacteria, thus proving by a recognized procedure that this nematode is the prime etiological agent.

It was observed that when the nematodes were added to a petri dish containing a root culture of either corn or tobacco, they were immediately attracted to the root. This attraction has been observed by other workers (Byars, 1914) and it is assumed that diffusible substances exuding from the root incite a positive chemotropic response on the part of the nematode. Upon reaching the root, many nematodes immediately attempted to enter the root tissues. Others, however, proved to be extremely lethargic, probably as a result of the sterilizing treatment, and did not attempt to penetrate the roots. In many cases, no penetration occurred at all, and the nematodes appeared to be feeding upon the root hairs. All such nematodes died within a period of from two to three weeks.

In those cases where penetration was successful, no living nematodes could be observed about the roots for a period of four to five weeks. Then, almost overnight, many nematodes of all stages could be seen in the agar about the root, and, following this initial appearance, nematodes continued to emerge

constantly. These, in turn, would attack new growth and soon the population would be increasing at a high rate. Most of the nematodes which emerged, however, did not re-enter the same root culture and, if a fresh culture were not introduced, they soon died. On several occasions, an active culture, such as described above, became somewhat dry and the nematodes in the agar inactive. Upon transfer of the root culture to fresh agar, hundreds of nematodes would emerge from the roots in a seething, sinuous stream, but, unless a fresh root culture were added at this time, most of them would be dead within a few days.

In general, only about 30% of the attempted cultures were successful. It is felt that the low percentage of successful inoculations is due to the nematodes being so weakened as a result of the sterilizing procedure that they are unable subsequently to penetrate the root epidermis.

Once the nematode cultures have become established, new cultures may be started by simply placing a fresh root tip in the culture for two to four days, and then transferring it to another petri dish. The roots were generally transferred to fresh medium every two weeks and cultures have been kept in this way for four months, at which time root growth had ceased, although root cultures appeared to be living.

The main difficulty with this technique at the present time is preventing contaminants becoming established on the agar. It is very difficult to keep a petri plate culture free of contaminants longer than two weeks if the plates are examined daily. It is hoped to evolve a new type of container which will solve this problem.

Summary

A technique of rearing plant parasitic nematodes under aseptic conditions on root tissue cultures is described. The application of this to the study of nematode diseases of plants should be very useful.

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A Method for Mass Recovery and Hatching of Nematodirus Eggs*

LEE SEGHETTI

Fecal cultures are commonly resorted to for demonstrations and production of larvae of gastro-intestinal nematodes. While culturing feces from lambs carrying heavy pure infections of *Nematodirus* sp. by accepted methods, it was found impossible to obtain consistently large numbers of larvae for experimental purposes without encountering difficulties and expending much time.

Therefore, a method was devised which assured a continuous supply of larvae without difficulty. By this method, large quantities of feces can be handled in a minimum of time, and large numbers of eggs can be cultured and hatched easily. The procedure that was used to concentrate the eggs and hatch Nematodirus larvae is as follows:

1. Feces were collected over a 24-hour period in bags tied to sheep infected with Nematodirus, principally. *N. spathiger.*
2. The feces were broken up in water by means of an electric blendor (2) to form a fine suspension using approximately 250 grams of feces to 750 ml. of water.
3. Small quantities of this suspension were passed through an 8 inch, 25 or 40 mesh, U. S. Standard sieve, depending upon the character of the feces, into a container that easily accommodated the sieve. To minimize trapping of the eggs in debris on the surface of the screen, small amounts were poured through the screen at a time. The material on the screen was washed briefly with a stream of tap water and then the sieve was transferred to a similar vessel containing 4 to 6 liters of tap water. The sieve was moved up and down in the water with a rocking motion for 5 to 10 minutes, at the same time avoiding spillage of the material over the side of the sieve. This procedure was repeated in a third container. The filtrate in the 3 containers was combined and agitated. At this point the eggs were contained in a relatively large volume of water.
4. The filtrate was siphoned through an 8 inch, 100 mesh, U. S. Standard sieve into a suitable container. When the surface of the sieve screen became covered with fecal material the siphoning was stopped and the sieve transferred to a vessel containing several liters of tap water. The sieve was manipulated as described above and the material on the surface of the sieve screen was washed briefly with tap water.
5. The filtrate was then siphoned through a 200 mesh U. S. Standard sieve and the filtrate discarded. The material remaining on the surface of the screen was washed gently with tap water. Then the sieve was inverted over a large circular dish and the material washed with sufficient water to remove all of it from the screen surface. The remaining fluid and sediment in the dish yielded a concentration of the Nematodirus eggs. The openings in a number

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200 sieve are of sufficient size to permit the smaller eggs of other trichostrongylids, if present, to pass through the screen while the majority of the Nematodirus eggs are retained.

The concentrated mass of eggs was placed in a constant temperature water bath (figure 1) equipped with a continuous stirrer. Sufficient water was added to submerge the thermo-regulator. It has been found that 30° C. is the optimum temperature for development and hatching of Nematodirus eggs.

The eggs hatch in 8 to 10 days. After hatching is complete the contents of the water bath are distributed in several tall containers and placed in the refrigerator at 4° C. for 24 hours. The Nematodirus larvae settle to the bottom and the supernatant fluid is siphoned off to within 5 centimeters of the sediment.

Using other methods of fecal culture, Nematodirus eggs containing fully developed larvae can frequently be seen still unhatched after the eggs have incubated for 14 days at 26° to 30° C. By this method unhatched eggs containing viable larvae have seldom been observed. In no instance were any ruptured eggs noted as a result of mechanical agitation. As indicated by flotation of the sediment after hatching of the larvae has been completed, the unhatched eggs observed were non-fertile.

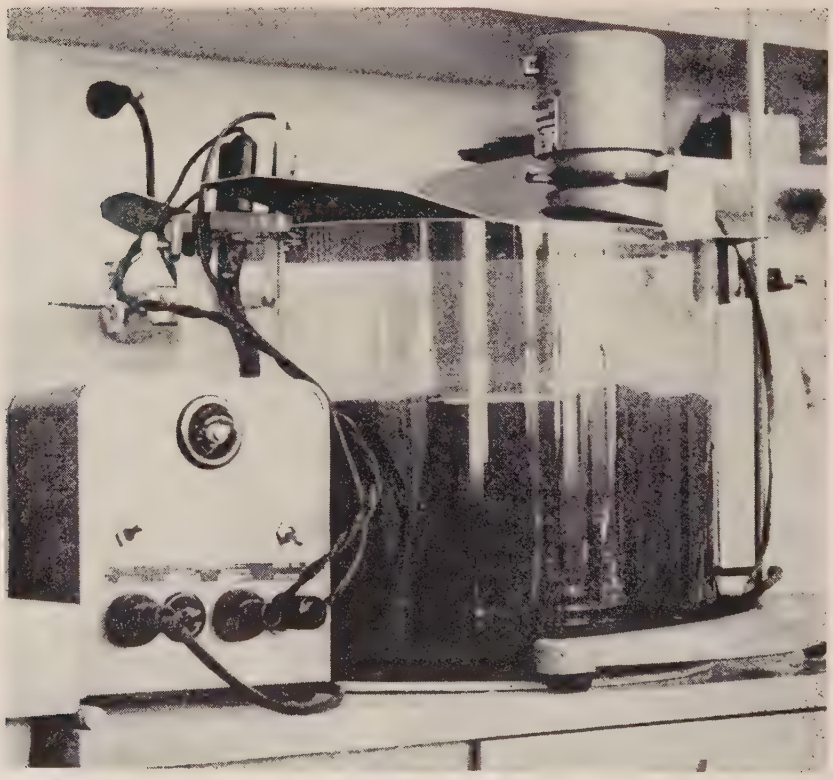

Figure 1. Constant temperature water bath used for incubation and hatching of *Nematodirus* eggs.

In a trial comparing the hatching of Nematodirus eggs placed in shallow petri dishes, containing 3 to 5 mm. of water, and maintained in a constant temperature incubator at 30° C., with the technic described, a 15% better hatch was obtained by the latter method.

Essentially the above technic, except for using sieves of closer mesh, has been used successfully in concentrating and sporulating large numbers of coccidial oocysts of ruminants.

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Errata

In the article by Bosher and McKeen (Proc. Helm. Soc. Wash. 21:113-117. 1954) three typographical errors occurred in the last three lines of the body of the table on p. 116. Those three lines *corrected* are as follows, the actual corrections being *italicized:*

Wool in water 10 minutes	dispersed		*trace*	trace
Wool in water 2 minutes	*partly dispersed*		70	40
Wool only			*85*	80

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